Aus der Urologischen Klinik und Poliklinik der

Ludwig-Maximilians-Universität München

Direktor: Prof. Dr. med. Christian G. Stief

Inhibition of human prostate smooth muscle contraction by the LIM kinase inhibitors, SR7826 and LIMKi3

Dissertation

zum Erwerb des Doktorgrades der Medizin

an der Medizinischen Fakultät der

Ludwig-Maximilians-Universität zu München

vorgelegt von

Qingfeng Yu

aus

Guangdong, China

2018

Mit Genehmigung der Medizinischen Fakultät
der Universität München

Berichterstatter: Prof. Dr. med. Christian. G. Stief

Mitberichterstatter: Priv. Dr. Claudia Veigel
Priv. Dr. Oliver Reich

Mitbetreuung durch den
Promovierten Mitarbeiter: Priv. Doz. Dr. rer. nat. Martin Hennenberg

Dekan: Prof. Dr. med. dent. Reinhard Hickel

Tag der mündlichen Prüfung: 19.04.2018

Contents

1 Introduction

1.1 Definition

Lower urinary tract symptoms (LUTS) refer to a group of urological symptoms which can be categorized into storage (syn. irritative) and voiding (syn. obstructive) symptoms. The voiding/obstructive symptoms include poor or intermittent stream, hesitancy, terminal dribbling, incomplete voiding, overflow incontinence and urinary retention (UR). The storage/irritative symptoms appear as increasing frequency or urgency of urination, nocturia, and could also be painful during urination (Gratzke et al., 2015).

LUTS were once emphasized to be mainly caused by benign prostatic hyperplasia (BPH), however, recent studies found that LUTS were often not just related to BPH (Abrams, 1994; Chapple et al., 2008), and increased evidences indicated and clarified the understanding of the LUTS as a functional unit, and the multifactorial etiology could be responsible for associated and diverging symptoms. In fact, recent studies have demonstrated that bladder dysfunction including detrusor overactivity (DO) and detrusor underactivity (DU), functional or structural abnormalities of urinary tract and surrounding tissues may cause LUTS (Drake, 2014; He et al., 2016). In addition, some non-urological disorders such as metabolic syndrome may also represent etiologies of LUTS (Bergdahl et al., 1998; Koskimaki et al., 2001; McDonald et al., 2017).

1.2 Epidemiology

LUTS are common among both female and male patients, while prevalence

reported from epidemiological studies differed, ranging from 13% (Boyle et al., 2003b) to 67% (Lee et al., 2011). It is widely accepted and indicated from most of studies that the prevalence of LUTS increases with age (Coyne et al., 2009; Irwin et al., 2006; Irwin et al., 2009; Irwin et al., 2011; Stewart et al., 2003). Nearly 10% of male and 11% of married females aged 40-49 years reported moderate or severe symptoms, while at the age of 70-79 years old, the percentages increased remarkably to 40% and 28% for male and female, respectively (Boyle et al., 2003b). Another recent study on male population also reported that 40% of men over the age of 40 complained of moderate to severe LUTS, and 70% of men above the age of 70 years, while the proportion of men suffered from LUTS increased to 90% in cohort over the age of 85 years (Hollingsworth and Wilt, 2014). Older male population has a higher incidence of LUTS than older female (Boyle et al., 2003a; Irwin et al., 2011), approximately 2/3 of elderly men in a previous studied population reported to suffer at least one LUTS complaint (Garraway et al., 1991; Nielsen et al., 1994).

As the predicted aging of the worldwide population, the future worldwide prevalence is expected to increase accordingly. It was estimated that voiding symptoms will affect more than 600 million men in 2018 worldwide, which will cause high economic burden for the society (Irwin et al., 2011; MacKenzie and Aning, 2016; Taub and Wei, 2006). Regarding any LUTS, i. e. voiding and irritative symptoms, even around 1.5 billion in men, and 3.1 billion patients may be expected in 2018 for both genders (Irwin et al., 2011).

1.3 Etiology

Multifactorial etiology contributes to associated symptoms. It is widely accepted that aging might be one of the most important factors for LUTS

(Vahabi et al., 2017). Figure 1 shows several further potential causes of LUTS. Distal ureteric stones or stricture could cause partial obstruction in ureter during voiding, which is characterized by difficulty in voiding and reduced urine flow rate.

BPH, which causes benign prostate obstruction (BPO) and bladder outlet obstruction (BOO), is responsible for symptoms such as dysuria, painful urination, hesitancy, straining, splitting and spraying, intermitted stream, terminal dribbling, incomplete voiding, overflow incontinence, feeling of incomplete emptying, post-micturition dribbling, and finally urinary retention (UR) (Abrams et al., 2002; Chapple, 2011). Altogether, voiding symptoms suggestive of BPH are caused and characterized by a lowered urinary flow (Q_{max}) (Roehrborn, 2008). It is widely accepted, that increased prostate size due to hyperplastic growth on the one hand, and an increased smooth muscle tone in the hyperplastic prostate may both cause compression of the urethra, which impairs bladder emptying and urinary flow and results in symptoms (Cioanta and Muschter, 2000; Guneyli et al., 2017; Hennenberg et al., 2014b; Oelke et al., 2015).

In parallel or even in addition to LUTS suggestive of BPH, irritative symptoms due to overactive bladder (OAB) are common. These are caused by involuntary detrusor contractions, which will result in symptoms including increased daytime frequency, nocturia, urgency, urinary incontinence (Apostolidis et al., 2017; Chapple, 2011; Izett et al., 2017; Lee and Kuo, 2017a). The origin of these contractions may be neurogenic, but may also include other factors such as spinal cord injury, prostate surgery or external radiation of prostate cancer (Kowalczyk, 2003). LUTS caused by chronic pelvic pain syndrome could present symptoms as dysuria, increased urgency of urination or painful urination but without evidence of urinary tract infection (Doggweiler et al., 2017).

Bladder tumor that locates in bladder outlet might cause BOO and is characterized by increased detrusor pressure and reduced urine flow rate. Detrusor underactivity (DU) may also cause difficulties in voiding and chronic UR, what may be difficult to distinguish from BOO (Gratzke et al., 2015).

Besides, some other diseases have been found to be risk factors for LUTS, for example, nocturnal polyuria, which is characterized by increased excretions of water and sodium during sleeping time but normal excretions in daytime. It is speculated that nocturnal polyuria and natriuresis are responsible for most nocturia in elderly men with LUTS (Matthiesen et al., 1996).

Foreign body, most observed in bladder, have been reported to cause LUTS, and most of these patients might present urinary tract infection or painful urination (Bansal and Yadav, 2016). Besides, non-urogical disease such as Parkinson's disease (McDonald et al., 2017), drug abuse (Peng et al., 2014), metabolic disorders (Hammarsten and Peeker, 2011), and arthritis (Koskimaki et al., 2001) have been also suspected to associate with LUTS.

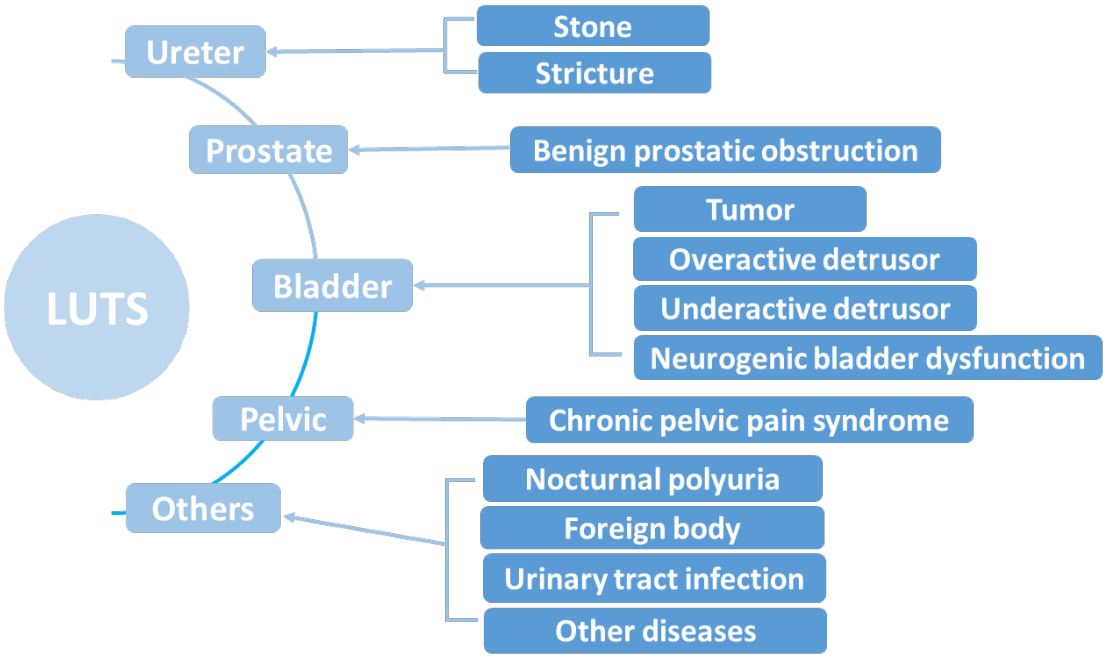


Figure 1: potential causes of LUTS (Gratzke et al., 2015). LUTS may be primarily caused by the prostate and the bladder. Other factors may cause LUTS as well, which need to be considered particularly during diagnosis of LUTS.

1.4 The role of BPH in LUTS

Although it becomes increasingly clear that BPH is not the single contributor to LUTS, it remains one of the most crucial factors. In fact, BPH could be observed in the majority of elder men and might then cause LUTS (Milani and Djavan, 2005). Considering the expected demographic transition in Western and Asian countries with a growing population of elderly men, the importance of LUTS suggestive of BPH will increase in the near future (Lepor, 2005).

The age of initiation of the growth of BPH was estimated to be from 30 years and upwards. Nearly 50% of men >50 years old will present pathological evidence of BPH, and the percentage was supposed to increase to more than 80% in the groups of patients with an age of 80 years old or older (Berry et al.,

1984). In fact, approximately every man at some point in his life will have LUTS secondary to BPH (Lepor, 2005).

The pathophysiology of LUTS is considered to include BOO (bladder neck abnormity, hyperplastic prostate or urethral stricture), or bladder dysfunction (DU or DO) (Lee and Kuo, 2017b). The pathophysiology of BOO in men with BPH consists of static and dynamic factors (Lepor, 2005). The static obstruction means that the hyperplastic prostate may cause compression of both the urethra and bladder outlet which could result in increased resistance to urine flow (Hennenberg et al., 2014a). The dynamic obstruction is due to an increased tension of prostate smooth muscle, which causes obstruction of the urethra (Hennenberg et al., 2014b).

It is widely recognized that activation of α_1-adrenoceptors in the prostate causes smooth muscle contraction, which contributes to increases smooth muscle tone and BOO in BPH (Hennenberg et al., 2014b). Previous studies have reported that the density of α_1-adrenoceptors was significant higher in prostate tissues from patients with BPH than those without (Ishigooka et al., 1997; Kondo et al., 1993; Price et al., 1993; Walden et al., 1999), suggesting that enlarged prostates can reduce the urine flow rate via increased responsiveness to α1-adrenergic prostate smooth muscle contraction.

It is widely accepted that the amount of prostate smooth muscle contributes to BOO, and that the improvement in LUTS suggestive of BPH by α_1-blockers might be related to relaxation of prostate smooth muscle (Hennenberg et al., 2014b; Lepor, 2005; Oelke et al., 2014). Consequently, α_1-blockers are the first line option for treatment of LUTS suggestive of BPH (Hennenberg et al., 2014a; Oelke et al., 2012a). They will induce smooth muscle relaxation in the prostate and thereby reduce voiding symptoms.

1.5 Pharmacological treatment for LUTS

1.5.1 Current therapies

α_1-adrenoceptor antagonists (α1-blockers)

α_1-blockers are considered to be the medication showing fastest reduction of LUTS secondary to BPH. The current α_1-blockers available for clinical use are tamsulosin, alfuzosin, terazosin, doxazosin, which are similar in overall clinical impact (Djavan et al., 2004a; Milani and Djavan, 2005). α_1-blockers can reduce the prostate tone and BOO via inhibiting the effect of endogenously released noradrenaline on prostate smooth muscle cells. Previous studies showed that α_1-blockers increased the Q_{max} by around 20-25% and reduced International Prostate Symptom Score (IPSS) by around 30-40% (Djavan et al., 2004b; Kang et al., 2011; Manjunatha et al., 2016; Michel et al., 1998; Seo and Kim, 2012). α_1-blockers can reduce both voiding and storage symptoms, and the efficacy seems to be more remarkable in those patients with prostates less than 40 mL (Roehrborn et al., 2008b; Roehrborn et al., 2010). α_1-blockers take only a few weeks to fully develop the efficacy and show well tolerability and safety during the treatment (Michel et al., 1998).

5α−reductase inhibitors (5−ARIs)

Testosterone is converted to dihydrotestosterone (DHT) mediated by the enzyme 5α-reductase and thereby has its effects on the prostate, to cause prostate hyperplasia and LUTS (Andriole et al., 2004). 5-ARIs inhibit the activation of 5α-reductase and induce apoptosis of prostate epithelial cells (Rittmaster et al., 1996). The current 5-ARIs available for clinical use are finasteride, dutasteride and epristeride. Prostate size was observed to be reduced by approximately 18-28% by these compounds, and levels of circulating prostate-specific antigen (PSA) were decreased by approximately

50% after six to twelve months of treatment with 5-ARIs (Vaughan, 2003). These effects could be more remarkable after long-term treatment (Naslund and Miner, 2007). 5-ARIs improve IPSS by around 15-30% and increase Q_{max} by around 1.5-2.0 mL/s after two to four years of treatment for those patients with LUTS due to BPH (Kirby et al., 2003; McConnell et al., 1998; Roehrborn et al., 2002). Also, 5-ARIs reduce either the risk of AUR or the need for surgery (Roehrborn, 2008).

Muscarinic receptor antagonists

Muscarinic receptors locate on bladder smooth muscle cells. They are activated by acetylcholine, causing contraction of the detrusor. Consequently, muscarinic receptor antagonists are used to reduce detrusor smooth muscle contraction in order to treat OAB due to DO (Hennenberg et al., 2014a; Hennenberg et al., 2017b). The current muscarinic receptor antagonists licensed for clinical use are tolterodine, solifenacin, propiverine, oxybutynin, fesoterodine, and darifenacin. Antimuscarinics can significant reduce urgency episodes, urgency incontinence, frequency of micturition and nocturia in patients with OAB (Herschorn et al., 2010; Kaplan et al., 2010; Roehrborn et al., 2006; Yokoyama et al., 2009). More benefit was observed in men with smaller prostates (PSA levels <1.3 ng/mL) from receiving antimuscarinics (Roehrborn et al., 2008a).

Antimuscarinics might decrease the strength of bladder detrusor, and increase the post-void residual (PVR) urine in men with mild to moderate BOO (Abrams et al., 2006). Also, the increased risk of UR after treatment of antimuscarinics should be considered from the theoretical view.

Putative novel muscarinic receptor antagonists have been explored in preclinical studies. Izalpinin which was exracted from Alpinia oxyphylla, showed an inhibitory effect in muscarinic detrusor contraction in vitro (Yuan et

al., 2014). Mokry et al. reported that propantheline significantly decreased the acetylcholine-induced smooth muscle contraction of guinea pigs urinary and bladder, while the inhibiting effects revealed a little less effective compared with oxybutynin (Mokry et al., 2005).

Phosphodiesterase (PDE) inhibitors

PDE inhibitors (PEDIs) can reduce the smooth muscle tone in the urethra, prostate, and detrusor via increasing intracellular cyclic guanosine monophosphate (cGMP). The current PDEI licensed for treatment of LUTS is tadalafil. Efficacy of PDE5Is could be observed one week following initiation of treatment with a reduction of IPSS by 22-37% (Oelke et al., 2012b), and after four weeks of treatment with the PDE5Is, approximately 79.3% of studied men were reported to have clinical meaningful improvement (Oelke et al., 2015). PDE5Is reduces smooth muscle tone and significant increases Q_{max}, but shows not any effect on increasing the risk of PVR (Porst et al., 2013).

Other PDE isoforms have been also explored. TC-E 5005, a phosphodiesterase 10-selective inhibitor, was found to inhibit phenylephrine-, U46619-, endothelin 1-3-induced, concentration-dependent contractions as well as electric field stimulation (EFS)- induced, frequency-dependent contractions of prostate strips in vitro (Hennenberg et al., 2016). PDE 4 inhibitors (PDE4Is), Ro 20-1724, rolipram, and RP 73401 also exhibited inhibiting effects on the tension induced by norepinephrine of isolated prostatic tissue, and the efficacy turned out to be stronger than PDE5Is, sildenafil and BAY 13-1197 (Kedia et al., 2012). Reversion of smooth muscle tension with various inhibiting effects could be observed with the PDE1 inhibitor Vinpocetine, the PDE2 inhhibitor EHNA, and PDE4 inhibitor rolipram (Kedia et al., 2009).

β_3-adrenoceptor agonists (β_3-agonists)

β_3-adrenoceptor agonists can induce detrusor relaxation of bladder via stimulating the β_3-adrenoceptors which are expressed in smooth muscle cells of the bladder (Afeli et al., 2013; Alexandre et al., 2016). The current β_3-adrenoceptor agonist approved for clinical use in adults with OAB is mirabegron. β_3-adrenoceptor agonists can significant reduce the symptoms of OAB, including frequency of micturition and urgency incontinence. Comparing with placebo, β_3-adrenoceptor agonists showed no remarkable adverse effects on voiding urodynamic parameters such as Q_{max}, bladder contractility index and detrusor pressure of maximum flow. Therefore, the risk of increased PVR after treatment of β_3-adrenoceptor agonists might be little (Nitti et al., 2013; Takeda et al., 2003). However, the efficacy and safety of β_3-adrenoceptor agonists in long-term therapy remain unclear (Hennenberg et al., 2014a; Michel and Gravas, 2016).

Some novel selective β_3-adrenoceptors have been reported. A recent study showed that 138-355 could cause bladder detrusor relaxation in vitro via stimulating β_3-adrenoceptor (Yamanishi et al., 2006). Another new compound, GW427353, was observed to relax detrusor at low concentrations (<10μM) and to suppress spontaneous detrusor contraction at higher concentrations (>50μM) (Biers et al., 2006).

Limits of current therapies

α_1-blockers remarkably reduce the IPSS and increase Q_{max}, with a reduction of IPSS by 30-40% and increases of Q_{max} of 20-25% (Djavan et al., 2004b; Hennenberg et al., 2014a). However, considerable effects of placebo were also found in the control group, which showed an IPSS improvement by approximately 10–34%, and an Q_{max} increase up to 27% (Hennenberg et al.,

2014b). α_1-blockers show no effect on reducing the prostate volume, preventing the occurrence of acute UR or the need for surgery (Roehrborn, 2006). As to 5-ARIs, it might only be considered in men with moderate to severe LUTS, for patients with prostates size < 40 mL, 5-ARIs might not be more efficacious than placebo (Boyle et al., 1996). Besides, the onset of efficacy of 5-ARIs is quite slow (usually requires three months), and monotherapy is not effective enough for patients with moderate-to-severe LUTS after initiation of therapy (Naslund and Miner, 2007).

This disappointing efficacy may be responsible for high discontinuation rates, which is associated with LUTS treatment by α_1-blockers: thus, one year after first prescription of α_1-blockers, only 35 % of patients still continued medication (Cindolo et al., 2015; Lee et al., 2015). Up to 69 % of patients may not be satisfied by treatment with α_1-blockers, and still 36-45 % are even unsatisfied by combination therapy of α_1-blockers with 5-ARIs (Fullhase et al., 2013). Indeed, insufficient efficacy of α_1-blockers is a main reason to change to another medication (Kim et al., 2014). This reflects low responder rates: in 30-35 % of patients, improvements in total IPSS by α_1-blockers are below 25 % (Chapple et al., 2011; Matsukawa et al., 2013). Regarding Q_{max}, responder rates may be even similar under treatment with α_1-blockers and placebo (Chapple et al., 2011). Finally, low adherence to medical LUTS therapy was identified as an independent risk factor for hospitalization for BPH and for surgery (Cindolo et al., 2015). Together, this demonstrates, that the efficacy of α_1-blockers underlies certain limits, despite their widespread application, so that new options with higher efficacy are mandatory (Hennenberg et al., 2014b).

PDE5Is were recommended to treat LUTS in recent years, however, their tolerability and safety in long-term use (> 1 year) remain unclear, and their effects on reducing prostate size and slowing disease progression are still needed to be identified. Although combination therapies such as α_1-blockers +

5-ARIs, or α_1-blockers + muscarinic receptor antagonists have been recommended, the increase of relevant adverse effects should be also considered (Gratzke et al., 2015).

Plant extracts

Herbal drugs are made of seeds, pollen, roots, bark, or fruits. Up to date, a growing number of plant extracts, such as izalpinin and phellodendron amurense have been reported to show effects on reducing smooth muscle contraction of either bladder (Brandli et al., 2010; Capasso et al., 2004; Fouda et al., 2007; Furer et al., 2015; Ito et al., 2009; Kim et al., 2003; Levin et al., 2002; Onoruvwe et al., 2001) or prostate (Arruzazabala et al., 2006; Brandli et al., 2010; Xu and Ventura, 2010) from animals (rat, rabbit, or pig). A possible mechanism on modulating Ca^{2+} release from intracellular stores was demonstrated to be responsible for the inhibitory effect of the flavonoid galangin (Dambros et al., 2005), however, the precise mechanisms of most reported plant extracts are still unclear, and the confirmation of their effects in vivo are warranted.

1.5.2 Novel compounds in pathophysiology and experimental therapy of LUTS

Current pharmacological therapies available for LUTS are characterized by limited efficacy, and the disappointing effects may cause high discontinuation rates of patients. This makes medical treatment of LUTS still be a challenge, therefore development of novel pharmacological options is still quite required (Chapple et al., 2011; Fullhase et al., 2013; Hennenberg et al., 2014b; Lee et al., 2015; Matsukawa et al., 2013). In fact, in recent years, a number of novel compounds have been found to be possible potential candidate therapy options

for LUTS, and some of them are to be further explored for clinical application.

$P2X_1$-purinoceptor antagonists

Adenosine 5'-triphosphate (ATP) was demonstrated as an excitatory cotransmitter with noradrenaline from sympathetic nerves which innervates the prostate smooth muscle contraction of guinea-pig (Buljubasich and Ventura, 2004) and rat (Ventura et al., 2003). 50-fold greater potency of endogenous purinoceptor agonist ATP was observed in aged mice than young adult mice, and nerve-mediated smooth muscle contraction containing a component elicited by P2X1 purinoceptor could only be observed in prostates from aged mice, but not from young adult mice (White et al., 2015).

In vitro experiments showed that P2X1-purinoceptor selective antagonists prevented augmentation of rat detrusor contractile response induced by hypoxia-glucopenia and reoxygenation (Elliott et al., 2013), which means P2X1-purinoceptor antagonists might be a possible future therapeutic option for increased purinergic-mediated detrusor contractions.

Cannabinoids agonist

Previous studies have indicated the importance of the endocannabinoid system in LUT dysfunction (Pacher et al., 2006). A cannabinoid agonist, WIN 55212-2, has been shown to inhibit electric field stimulation-induced contractions of rat prostate smooth muscle (Tokanovic et al., 2007). In human prostatic strips, CP 55940, a cannabinoid receptor 1/cannabinoid receptor 2 agonist, also showed inhibiting effects on nerve-induced contractions (Gratzke et al., 2010). Later studies also reported that cannabidiol was observed to reduce acetylcholine-induced smooth muscle contraction both from rat and human prostates (Capasso et al., 2011). Although cannabinoids agonists are now

proved to be effective on reducing contractility, the inhibitory effects seemed to be only modest (Gratzke et al., 2010; Tokanovic et al., 2007).

Prostaglandin pathway regulators

There are mounting evidences that inflammation may act as a risk factor for the development of BPH and LUTS (Kramer et al., 2007). Prostaglandin (PG) E_2 has been observed to reduce contractile response of rat prostate gland via acting at EP_2 subtype (Tokanovic et al., 2010). A recent study showed that MF191, an antagonist of PGE_2 receptor subtype 4 (EP_4), has significant effects on cyclophosphamide or PGE_2-induced overactive bladder in rats (Chuang et al., 2012). ONO-8539, an antagonist of PGE_2 receptor subtype 1 (EP_1), showed significant efficacy, safety and tolerability for patients with OAB in a phase II study (Chapple et al., 2014).

PG-caused LUTS symptoms could be released by other non-PG inhibitors. Latest studies have found that fatty acid amide hydrolase (FAAH) inhibitors, transient receptor potential melastin 8 ion channel (TRPM8) -selective antagonist (DFL23448) and oleoyl ethyl amide (OEtA), exhibited effects on increasing micturition intervals, micturition volumes, and bladder capacity in rat presented OAB induced by PGE_2 (Gandaglia et al., 2014; Mistretta et al., 2016). However, the use of PGs for LUTS might be limited when considering their widespread effects throughout the whole body.

Cyclooxygenase (Oelke et al.), also known as prostaglandin-endoperoxide synthase (PTGS), is an enzyme responsible for the formation of PG. Diclofenac and NS-398 have been demonstrated to reduce serotonin, noradrenaline, and neurokinin A-induced isolated ureter contractions from pig in a concentration-dependent manner via inhibiting COX subtype 2 (Mastrangelo et al., 2000). A later study reported that indomethacin, a non-selective COX inhibitor, also

showed remarkable effect on reducing spontaneous detrusor contraction from isolated bladder of pig (Rahnama'i et al., 2013). The antibiotic erythromycin showed inhibiting effects on reducing rat detrusor contractile response induced by EFS, carbachol, ATP, and potassium via inhibiting the voltage-sensitve calcium channels and releasing calcium from intracellular stores (England et al., 2004).

Protein kinases (PK)

It has been well established that prostate smooth muscle contraction is linked to a number of intracellular signaling pathways under physiologic and pathophysiologic conditions, which include several protein kinases and GTPases (Hennenberg et al., 2014b). It has been demonstrated that dysfunction of Protein kinase C (PKC) is implicated to cause smooth muscle disorders. It has been shown that low concentrations of PKC inhibitors, bisindolylmaleimide1 (Bim-1) and Ro318220 (both in 50 nM), and a PKC activator, phorbol-12,13-dibutyrate (PDBu) (1 μM) reduced the sensitivity of detrusor to carbachol and decreased spontaneous contractions (Hypolite et al., 2015).

RhoA couples to excitatory receptors and activates Rho kinase, which further inhibits myosin light chain phosphatase and maintains a contracted state of actin filaments (Hennenberg et al., 2006; Somlyo and Somlyo, 2004). The RhoA/Rho kinase pathway has been explored using novel compounds which are emerging. Y-27632, a specific inhibitor of Rho kinase, attenuated the spontaneous and EFS-induced smooth muscle contractions of human ureteral strips in dose-dependent manners (Hong et al., 2005). Similar effects were also observed in KCl-induced ureter contractions from rabbits with unilateral ureteric obstruction (Turna et al., 2007). Y-27632 inhibited carbachol-mediated contractile responses in mouse prostate gland in low concentration (10 μM),

and inhibited noradrenaline-mediated contractile responses in high concentration (10-30 μM) (White et al., 2013). In human detrusor, Y-27632 and another Rho kinase inhibitor, HA1100, both inhibited carbachol-induced contractions in the concentration of 10 μM (Kirschstein et al., 2014). The RhoA/ROCK-mediated Ca^{2+} sensitization was also reported to involve in the human prostate contraction. Contraction of human prostatic stromal cells induced by endothelin-1 could be enhanced by over-expressed RhoA, but decreased by Y-27632 or fasudil. In human prostate strips, the noradrenaline-induced contraction could also be inhibited by Y-27632 or fasudil (Takahashi et al., 2007). Finally, Y-27632 also inhibited thromboxane-induced contractions of human prostate strips, besides α_1-adrenergic contractions (Strittmatter et al., 2011).

P21-activated kinase (PAK) acts together with Rac, which involves in a wide range of cellular functions such as cytoskeleton organization, smooth muscle contraction, and neuronal functions (Wang et al., 2016b). The PAK inhibitors FRAX486 (30 μM) and IPA3 (300 μM) have been observed to significantly inhibit prostate smooth muscle contraction induced by EFS, but not by noradrenaline or phenylephrine (Wang et al., 2016b). Besides, FRAX486 also showed inhibiting effects on endothelin-1- and -2-induced contractions (Wang et al., 2016b). Reduced growth of prostate stromal cells (WPMY-1 cells) was observed by both inhibitors at the same concentrations (Wang et al., 2016b).

Rac is a small monomeric GTPase which is involved in the regulation of smooth muscle tone via cytoskeletal organization (Wang et al., 2015). The Rac inhibitors NSC23766 (100 μM) and EHT1864 (100 μM) have been found to significantly reduce prostate smooth muscle contractile responses induced by noradrenaline, phenylephrine and EFS (Wang et al., 2015). Both inhibitors revealed effects of cytoskeletal disorganization and impairment in the growth of WPMY-1 prostate cells in these concentrations (Wang et al., 2015).

Src family kinases (SFKs) are tyrosine kinases involved in various key cellular functions such as rearrangement of actin structures via cycling in and out of focal adhesions (Min et al., 2012). A recent study showed that two SFK, AZM475271 and, reduced prostate smooth muscle contractile responses to noradrenaline-, phenylephrine- and EFS in vitro. Stronger inhibiting effect could be observed in combination of PP2 and PF573228 (a focal adhesion kinase inhibitor) when compared to PP2 alone (Wang et al., 2016a). Reduced proliferation in wildtype of WPMY-1 cells after incubation with each inhibitor could be observed but not in cells with c-Src-deficiency(Wang et al., 2016a). Finally, first evidence for a role of focal adhesions for promoting prostate smooth muscle contraction was derived by a study, showing that inhibitors of focal adhesion kinases inhibit adrenergic and neurogenic contractions of human prostate strips (Kunit et al., 2014).

Other compounds

A growing number of other types of compounds are also emerging, which show varying effects on the contractility of ureter, bladder, or prostate gland in various species. A role of TRPA1 agonists in inhibiting electrically induced isolate-ureters contractions has been proposed (Weinhold et al., 2017). Blebbistain, a myosin II inhibitor, showed significant effect on reducing nerve-evoked detrusor overactivity in a rat model (Zhang et al., 2011). Considering the large amount of patients suffering from LUTS, the development of novel therapy targets aiming to induce smooth muscle relaxation in the prostate and other LUT regions would be quite necessary and promising.

1.6 **Mechanisms of prostate smooth muscle contraction**

It is widely accepted that an increase in the free intracellular calcium level is a precondition to active the smooth muscle contractile proteins, while calcium sensitization is important for the ability of smooth muscle cells to maintain a contractile response in the presence of submaximal intracellular calcium levels (Webb, 2003). Agonist receptors in prostate stroma are regarded as the triggers the contraction of prostate smooth muscle. The α_1-adrenoceptors (α_1ARs) belong to the G protein-coupled receptor (GPCR) superfamily which occur in α_{1a}, α_{1b}, and α_{1d}AR subtypes in prostate stroma; it was demonstrated that the function of α_{1a}AR predominates in the prostate smooth muscle (Andersson et al., 1997). Agonist such as noradrenaline or phenylephrine binds to α_1ARs and increases phospholipase C (PLC) activity, which produces two potent second messengers inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (Gandaglia et al.). IP_3 causes release of calcium (Ca^{2+}), while DAG activates PKC (Wang et al., 2014). The binding of Ca^{2+} and calmodulin results in activation of myosin light chain (MLC) kinase, which further phosphorylates the light chain of myosin to combine with actin and leads to cross-bridge cycling and smooth muscle contraction (Dougherty et al., 2014). When the PLC is activated, a small GTP-binding protein RhoA is also activated by Rho guanine nucleotide exchange factor (RhoGEF). RhoA could increase the activity of Rho kinase that leads to inhibition of myosin phosphatase and maintains the phosphorylation of light chain of myosin so that to promote the contractile state (Webb, 2003). Similarly, PKC promotes contraction also by inhibition of myosin light chain phosphatase (Hennenberg et al., 2008).

In addition to adrenoceptors, non-adrenergic mediators such as endothelin-1 (Hennenberg et al., 2014b) and thromboxane A_2 (TXA_2) (Strittmatter et al., 2011) are also demonstrated to promote smooth muscle contraction. Contractions by endotheline-1 and TXA_2 might be additive, and even exceed

α_1-adrenergic tone (Hennenberg et al., 2017a). Endothelin-1 is demonstrated to cause contraction of smooth muscle in two signaling pathways. After the binding of endotheline-1 and its receptor, the PLC/ diacylglycerol (Gandaglia et al.) /IP3 signaling pathway is activated. Meanwhile, the mitogen-activated protein kinase (MAPK) is also activated, which further phosphorylates the caldesmon to promote contractile state (Bouallegue et al., 2007). TXA_2 receptors are detected in prostate smooth muscle cells, contraction of prostate smooth muscle induced by TXA_2 may involve in Ca^{2+}/ calmodulin- and Rho kinase- dependent mechanisms (Strittmatter et al., 2011).

Recently, it has been proposed that effects of α_1-blockers stay incomplete due to contributions of non-adrenergic mediators, as these mediators may evoke contraction of prostate smooth muscle in parallel to α_1-adrenoceptors (Hennenberg et al., 2013; Hennenberg et al., 2014b). Not surprisingly, but importantly, contractions of these non-adrenergic mediators will not be inhibited by α_1-blockers, so that they may maintain prostate smooth muscle tone and urethral obstruction even under therapy with α_1-blockers (Hennenberg et al., 2016). Consequently, the benefit from α_1-blockers may be limited, as long as non-adrenergic contractions still promote smooth muscle tone alongside to α_1-adrenoceptors (Hennenberg et al., 2016). Probably, simultaneous actions of different mediators on prostate smooth muscle cells are the normal case in vivo. Therefore, it has been postulated, that novel strategies for future therapies with higher efficacy than α_1-blockers may only be successful, if they target adrenergic and non-adrenergic contractions at once (Hennenberg et al., 2016).

Several intracellular effectors have been discovered to closely involve the contraction of prostate smooth muscle. P21-Activated Kinase (PAK) was demonstrated to promote release of noradrenaline from sympathic neurotransmission to smooth muscle cells, which thereby activated

postsynaptic α_1-adrenoceptors to cause smooth muscle contraction. Meanwhile, PAK could be also activated by endothelin receptor A/B, and establishes intracellular signaling pathway including Ca^{2+}/PKC/Rho kinase to promote the contraction of prostate smooth muscle (Wang et al., 2016b). It was recognized that small G-proteins play a key role in smooth muscle contraction (Kitazawa et al., 1989). Rac belongs to the small G-proteins, three Rac isoforms (Rac1-3) were all detected in prostate tissues (Wang et al., 2015). It was reported that Rac has a key role in regulating cytoskeletal organization which is responsible for contraction (Zhou et al., 2010). Focal adhesion kinase (Nitti et al.) is demonstrated to be the principal requirement for the correct assemble of adhesome proteins to dense plaques which enables the attachment of cytoskeleton to membranes and of membranes to extracellular matrix, finally promoting prostate smooth muscle contraction (Hennenberg et al., 2014b; Kunit et al., 2014). Besides, FAK may be also involved in phosphorylation of Ca^{2+} channel (Gerthoffer and Gunst, 2001).

Together and regardless of upstream signaling pathways, there are three prerequisites, which are indispensable for smooth muscle contraction, comprising 1) phosphorylation of myosin light chains, 2) actin organization and polymerization to filaments, and 3) attachment of filaments to membranes and extracellular matrix by dense plaques (Walther et al., 2012). Actin filaments are essential elements of the cytoskeleton, changes in actin polymerization and reorganization are responsible for cellular migration and a prerequisite for smooth muscle contraction (Gunst and Zhang, 2008; Kirschstein et al., 2015). Although the actomyosin system and actin polymerization could be activated independently, the development of smooth muscle contraction requires activation of both processes (Gunst and Zhang, 2008). Effectors on cytoskeleton or actin, such as LIM domain kinase (LIMK) (Bernard, 2007), ARF6 (Herlemann et al., 2017), and heat shock protein (HSP) (Hedges et al., 1999) are the key point in most of the signaling pathways during

the contractile process.

1.7 The role of LIMKs

LIM kinases (LIMKs) are serine/threonine kinases, which occur in two isoforms, LIMK1 and LIMK2. LIMKs regulate actin polymerization through phosphorylation of the actin depolymerizing factors (ADF)/cofilin, and thereby cause filament organization and stress fibre formation (Bernard, 2007). It has been demonstrated that LIMKs act as key factors in regulating muscle contraction through various signaling pathways which mainly include Rho/ROCK/LIMK1/2, cAMP/PKA/LIMK1/2, Rac/PAK/LIMK1/2, and Cdc42/MRCK/LIMK2 pathways (Webb, 2003) (Figure 2).

Although LIMK have been reported to act as crucial regulators in cofilin phosphorylation and actin polymerization, as far as we can retrieve, studies on effects LIMK selective inhibitors or the role of LIMK during smooth muscle contraction have not yet been explored in any organ. This might probably because specific small molecular inhibitors were not available before, but now, as two LIMK inhibitors SR7826 and LIMKi3 are available, this enables us to evaluate their effects on contraction of prostate smooth muscle.

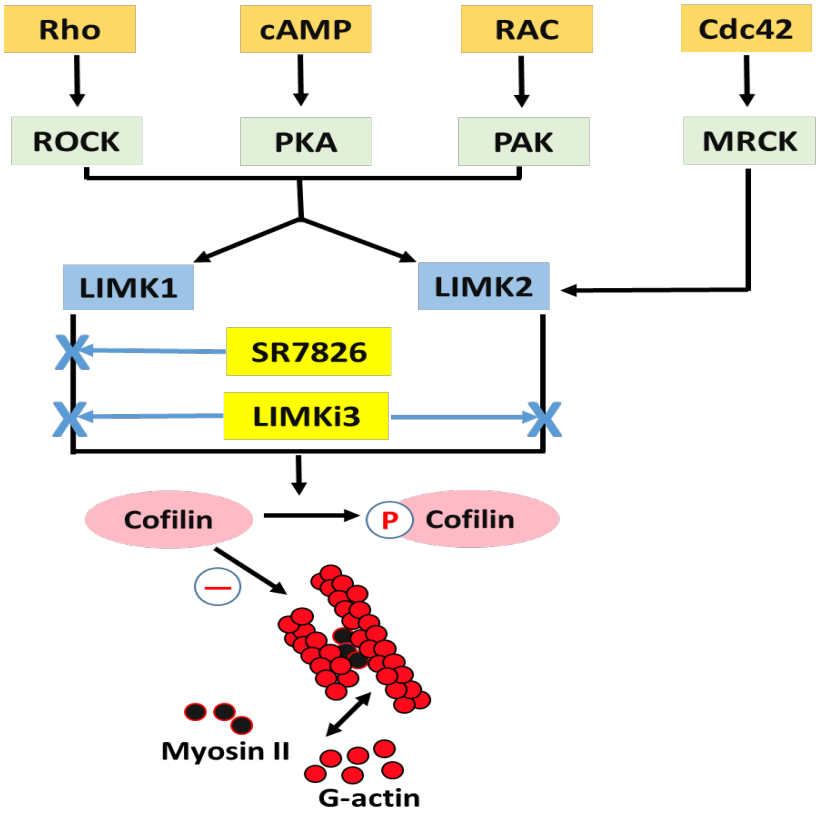


Figure 2: Assumed roles of LIMK1 and LIMK2 in smooth muscle contraction. LIMKs phosphorylate cofilin to phospho-cofilin, the inactive cofilin thereby causes smooth muscle contraction by actin polymerization. This process could be induced by several signaling pathways, e.g. Rho/ROCK/LIMK1/2, cAMP/PKA/LIMK1/2, Rac/PAK/LIMK1/2, and Cdc42/MRCK/LIMK2 pathways. Two LIMK inhibitors SR7826 and LIMKi3, are supposed to inhibit LIMK1 and LIMK1/2, respectively. Because actin polymerization is an indispensable prerequisite for smooth muscle contraction, a role of LIMK for promoting smooth muscle contraction may be assumed – but this has never been shown. Abbreviation: LIMKs, LIM domain kinases; Rho, rhodopsin; ROCK, Rho kinase; cAMP, Cyclic adenosine monophosphate; PKA, protein kinase A; RAC, ras-related C3 botulinum toxin substrate; PAK, p21-activated kinase; Cdc42, cell division cycle 42; MRCK, Cdc42-binding kinase.

2. Objective of this thesis

In BPH, smooth muscle contraction in the prostate drives urethral obstruction, which often results in LUTS. Consequently, inhibition of contraction is an important strategy for medical therapy, but available options still show insufficient efficacy. Development of future options with higher efficacy requires adequate understanding of contractile mechanisms and identification of novel targets in the prostate. However, despite the high clinical relevance of contractile mechanisms in the prostate, they are still incompletely understood. Recently, a possible role of LIMK has been suggested for actin polymerization in different cell types. Because actin polymerization is an ultimate prerequisite for smooth muscle contraction, a role of LIMK for regulation of smooth muscle tone may be assumed. Surprisingly and to the best of my knowledge, this has not been examined or reported to date. Therefore, the aim of this thesis was to study a possible role of LIMK for prostate smooth muscle contraction.

Specifically, the aim was to address the following questions:

(1) Are LIMKs expressed in the human prostate?

(2) Does the LIMK inhibitor SR7826 inhibit α_1-adrenoceptor-induced contractions of human prostate strips?

(3) Does the LIMK inhibitor SR7826 inhibit neurogenic contractions of human prostate strips?

(4) Does the LIMK inhibitor SR7826 inhibit non-adrenergic contractions of human prostate strips?

(5) Does the LIMK inhibitor LIMKi3 inhibit α_1-adrenoceptor-induced contractions of human prostate strips?

(6) Does the LIMK inhibitor LIMKi3 inhibit neurogenic contractions of human prostate strips?

(7) Does the LIMK inhibitor LIMKi3 inhibit non-adrenergic contractions of

human prostate strips?

(8) Is actin polymerization in prostate stromal cells susceptible to LIMK inhibitors?

3. Materials and methods

3.1 Reagents and devices

Table 1. Reagents used in this study.

Products	Manufacturer
Potassium chloride (KCl)	Roth, Germany
Sodium chloride (NaCl)	Roth, Germany
Calcium chloride dihydrate ($CaCl_2$•$2H_2O$)	Roth, Germany
Magnesium sulfate heptahydrate ($MgSO_4$•$7H_2O$)	Roth, Germany
Glucose	Roth, Germany
Sodium bicarbonate ($NaHCO_3$)	Sigma-Aldrich, USA
Potassium hydrogen phosphate (KH_2PO_4)	Applichem, Germany
Noradrenalin	Sigma-Aldrich, USA
Phenylephrine	Sigma-Aldrich, USA
Methoxamine hydrochloride	Sigma-Aldrich, USA
Custodiol	Köhler, Germany
U46619	Tocris, UK
Endothelin-1	Enzo, USA
LIMKi3	Tocris, UK
SR 7826	Tocris, UK
Dimethyl sulfoxide (DMSO)	Roth, Germany
RPMI 1640	Gibco, USA
10 % fetal calf serum (FCS)	Gibco, USA
phosphate-buffered saline (PBS)	Gibco, USA
Milk powder	Roth, Germany
Rabbit phospho-LIMK1 (thr508)/ LIMK2	Cell Signaling Technology, USA

(thr505) antibody	
Rabbit anti LIMK1 antibody	GeneTex, USA
Rabbit anti cofilin (D59)	Cell Signaling Technology, USA
Mouse anti p-cofilin 1	Santa Cruz Biotechnology, USA
Mouse monoclonal anti calponin 1/2/3	Santa Cruz Biotechnology, USA
Mouse monoclonal anti pan-cytokeratin	Santa Cruz Biotechnology, USA
Mouse monoclonal anti β-actin antibody	Santa Cruz Biotechnology, USA
Mouse monoclonal anti PSA antibody	Santa Cruz Biotechnology, USA
Horse anti mouse IgG	Vector Laboratories, USA
Horse anti goat IgG	Vector Laboratories, USA
Rabbit anti LIMK1 antibody	GeneTex, USA
Rabbit anti p-cofilin antibody	Cell Signaling Technology, USA
Rabbit anti cofilin antibody	Cell Signaling Technology, USA
Mouse anti pan-cytokeratin antibody	Santa Cruz Biotechnology, USA
Mouse anti calponin 1/2/3 antibody	Santa Cruz Biotechnology, USA
Cy5-conjugated goat anti rabbit IgG	Abcam, UK
Cy3-conjugated goat anti mouse IgG	Millipore, USA
Fluorescein isothiocyanate- conjugated Rabbit anti goat IgG	Millipore, USA
4’,6’-diamidino-2-phenylindole-dihydrochloride (DAPI)	Invitrogen, USA
Phalloidin	Sigma-Aldrich, Germany
MgCl2	Promega, USA
Revrse Transcription buffer 10×	Promega, USA
dNTP Mix	Promega, USA
Random Primers	Promega, USA
AMV Revrse Transcriptase	Promega, USA
RNase Inhibitor	Promega, USA
RNase Free Water	Promega, USA

SYBR™ Green	Roche,USA
RT2 qPCR Primer (LIMK1 & LIMK2)	QIAGEN, Germany
RNeasy Micro Kit	QIAGEN, Germany
AllPrep DNA/RNA/Protein Mini Kit	QIAGEN, Germany
BSA	Gibco, USA
5-Ethynyl-deoxyuridine (5-EdU)	Thermo Fisher Scientific, USA
Buffer additive CS219074	Thermo Fisher Scientific, USA
6-FAM-Azide	Thermo Fisher Scientific, USA
5/6-Sulforhodamine101-PEG3-Azide	Thermo Fisher Scientific, USA
5-TAMRA-PEG3-Azide	Thermo Fisher Scientific, USA
Eterneon-Red 645 Azide	Thermo Fisher Scientific, USA
10X Reaction Buffer	Thermo Fisher Scientific, USA
Triton X-100	Thermo Fisher Scientific, USA
Mounting solution	Thermo Fisher Scientific, USA
Dithiothreitol (DTT)	Sigma-Aldrich, Germany
Ethylenediaminetetraacetic acid (EDTA)	Sigma-Aldrich, Germany
Tris base	Sigma-Aldrich, Germany
LB agar	Thermo Fisher Scientific, USA
Tryptone	Sigma-Aldrich, Germany
Yeast extract	Sigma-Aldrich, Germany
GelRed	Thermo Fisher Scientific, USA
Agarose	Sigma-Aldrich, Germany
Film developers	Kodak, USA
Polyvinylidene-Fluoride (PVDF)	Sigma-Aldrich, Germany
chemiluminescence (ECL) Hyperfilm	GE Healthcare, Germany

Solutions used in organ bath were listed in Table 2.

Table 2: Composition of solutions used in organ bath.

Solution	Component	
Krebs- Henseleit solution 1 (KH1)	NaCl	172.4g
	KCl	8.8g
	$CaCl_2 \cdot 2H_2O$	9.2g
	KH_2PO_4	4.1g
	$MgSO_4 \cdot 7H_2O$	7.4g
Krebs- Henseleit solution 2 (KH2)	$NaHCO_3$	32.5g
Solution in Organ bath (Guirgis et al.)	Distilled water	1000ml
	KH-1	43.5ml
	Glucose	1.62g
	KH-2	43.5ml

Devices used in this study were listed in Table 3.

Table 3: Devices used in this study.

Products	Manufacturer
Tissue Bath System- 720MO	DMT (Danish Myotechnology), Denmark
Electrical Stimulation Fields	DMT (Danish Myotechnology), Denmark
Lab pump	KNF- Neuberger, USA
Thermostat	Memmert, Germany
American Type Culture Collection	ATCC Manassas, USA
Light Cycler PCR system	Roche, Switzerland
Protran® nitrocellulose membranes	Schleicher & Schuell, Germany
Gel electrophoresis system	Bio- rad, Germany
Superfrost® microscope slides	Thermo Fisher, USA
Laser scanning microscope	Leica SP2, Germany
Lab-Tek chamber slides	Thermo Fisher, USA
Cell culture incubator	Thermo Fisher, USA
Waterbath	Thermo Fisher, USA

3.2 Human prostate

Human prostate tissues were obtained from patients undergoing radical prostatectomy for prostate cancer (n= 123). Prostate tissues from those patients who previously received transurethral resection of prostate (TURP) or brachytherapy were excluded. All prostates were sent to the Department of Pathology immediately after prostatectomy and subsequently macroscopically examined by an experienced pathologist. Tissues were then taken from areas of

the periurethral zone, where no signs of neoplasia, cancer or inflammation were macroscopically observed.

The research was carried out according to the Declaration of Helsinki of the World Medical Association, and has been approved by the ethics committee of Ludwig-Maximillians University, Munich, Germany. Prostate tissues were either stored in Custatdiaol® at 4 °C for organ bath studies for a maximum of 90 min, or at -80 °C after shock frozen in liquid nitrogen for molecular research.

3.3 Cell culture

The cell line WPMY-1 used in this study which were purchased from American Type Culture Collection (ATCC Manassas, VA, USA), is an immortalized cell line from human prostate stroma without malignant transformation. Cells were recovered from liquid nitrogen and cultured in RPMI 1640 (Gibco, Carlsbad, CA, USA) supplemented with 10 % fetal calf serum (FCS) (Gibco, Carlsbad, CA, USA) and 1% penicillin (Gibco, Carlsbad, CA, USA)/ Streptomycin (Gibco, Carlsbad, CA, USA) at 37 °C in a humidified atmosphere with 5% CO_2. Cells were washed with PBS (pre-warmed to 37°C by water-bath) (Gibco, Carlsbad, CA, USA) and then incubated in 1 ml accutase (37 °C) for 5 min. After applying 10 ml of complete culture media to block the digestion process, cells were recollected by centrifugation (1,200 rpm, 5 min). Cell pellets were resuspended with culture media and transferred to flasks. Cells were detached after accutase digestion and centrifuged and resuspended in freezing media of 1ml (FBS with 10% DMSO), then transferred to a cryotube and stored at -80 °C for short-term or nitrogen tank for long-term storage.

3.3 Real time polymerase chain reaction (RT-PCR)

3.3.1 RNA isolation and concentration measurement

- For isolation from prostate tissues, the tissue amount was 30 mg. Tissues were homogenized performed with FastPrep®- 24 system with marix A (MP Biomedicals, IIIkirch, France). For isolation from prostate cells, the amount of cells was 1 x 10^7.
- Add Buffer RLT (600 µl) to prostate tissues or cells.
- Centrifuge the lysate at maximum speed for 3 min, then remove the supernatant by pipetting.
- Add 600 µl 70% ethanol to the lysate and mix completely by pipetting (no centrifuge in this procedure).
- Transfer 700 µl of the sample (including the precipitate) to the RNeasy Mini spin column placed in a 2 ml Eppendorf tube. Close the lid and centrifuge at maximal speed for 15 sec, and then discard the flow-through.
- Add 700 µl Buffer RW1 to the RNeasy spin column, centrifuge at maximal speed for 15 sec, and then discard the flow-through.
- Add 500 µl Buffer RPE to the RNeasy spin column, centrifuge at maximal speed for 15 sec, and then discard the flow-through.
- Add 500 µl Buffer RPE to the RNeasy spin column, centrifuge at maximal speed for 2 min, and then discard the flow-through.
- Place the RNeasy spin column in a new RNase- free collection tube (1.5 ml), add 50 µl RNase- free water to the spin column membrane, centrifuge at maximal speed for 1 min, and then collect the flow-through (repeat this procedure if necessary).
- Concentration measurement of RNA was performed with Nanodrop spectrophotometer, Nuclease- free water was used for blanking before measuring.

3.3.2 Reverse Transcription (RT)

RNA sample was incubated at 70 °C for 10 min, 1 µg of RNA sample was reverse transcribed using Reverse Transcription System (Promega, Madison, WI, USA) (Table 4).

Table 4: RT-PCR reaction system.

Components	Volumes (µl)
Random hexamer oligodeoxyribonucleotides	0.5
Ribonuclease inhibitor	0.5
Reverse-transcriptase (23 U/µl)	0.65
dNTP mix, 10 mM	2
10xRT buffer	2
$MgCl_2$, 25 mM	4
RNase-free water	10.35

3.3.3 Primer design

Based on the RefSeq accession numbers, NM_001204426 for LIMK1, NM_001031801 for LIMK2 and NM_002046 for Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), primers were provided by Qiagen (Hilden, Germany) as ready-to-use mixes.

3.3.4 Real-time polymerase chain reaction (RT-PCR)

RT-PCR for LIMK1, LIMK2 and GAPDH was performed with a Roche Light Cycler (Roche, Basel, Switzerland). PCR reactions were carried out in a volume of 25 µl which contained 5 µl LightCycler® FastStart DNA MasterPlus

SYBR Green I (Roche, Basel, Switzerland), 1 µl template, 1 µl primer, and 18 µl water. The settings run on the Light Cycler were used as followed:

Denaturation	**95°C**	**10 min**	
Denaturation	**95°C**	**15 sec**	**45 cycles**
Annealing and 3xtension	**60°C**	**60 sec**	

Melting curve

Cooling

ΔΔCP crossing points (CP) method was used to express the results (Livak and Schmittgen, 2001). ΔCP was defined as the value which number of cycles (Abrams et al.) for LIMKs subtracted the Ct values at which the fluroscence signal exceeded a defined threshold of GAPDH ($\Delta CP = Ct_{LIMK} - Ct_{GAPDH}$). Values were then calculated as $2^{-\Delta CP}$ and normalized to each other.

3.4 Western blotting

- Samples preparation:

  Fresh prostate tissues were cut into several small strips at the size of approximately 6 x 1 x 1 mm^3 and separated into two groups (control and inhibitor, or control and agonist, solvent was used as control). Tissues were placed in 6-well plates which filled with 10 ml custodiol®, and allowed to reach an equilibration period after 20 min before adding inhibitors (1µM of SR7826 or LIMKi3), agonist (30 µM of phenylephrine or U46619) or control (solvent). Plates were then under continuous shanking at 37 °C for incubation.

  For the detection of SR7826- or LIMKi3-induced cofilin phosphorylation, the incubation duration was 2 h, control groups were incubated with

solvent DMSO in same conditions. For the detection of agonist-induced cofilin and LIMK phosphorylation, the incubation durations were 10 min or 45 min for phenylephrine, or 1 h for U46619, control groups were incubated with solvent (water for phenylephrine, and ethanol for U46619) in same conditions. Tissues were then frozen with liquid nitrogen and stored at -80°C until needed.

- Homogenization of the prostate tissues in a buffer containing 10 µM phenylmethanesulfonyl fluoride, 10 µg/ml leupeptine hemisulfate, 1 mM benzamidine, and 25 mM Tris/HCl using a FastPrep®-24 system with matrix A (MP Biomedicals, Illkirch, France).
- Centrifugation of the homogenates at 20,00g for 4 min, transfer of the supernatant to a new Eppendorf Tubes.
- Assaying the protein concentration in supernatant using the Dc-Assay kit (Biorad, Munich, Germany) according to the manual instruction.
- Boiling the supernatant for 10 min with sodium dodecyl sulfate (SDS) sample buffer (Roth, Karlsruhe, Germany).
- Subjecting the samples (20 µg/lane) to SDS-polyacrylamide gel electrophoresis for separation of the proteins.
- Electroblotting of the proteins on Protran® nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany).
- Blocking the membranes with PBS containing 5 % milk powder (Roth, Karlsruhe, Germany) at 4 °C overnight.
- Incubation of the membranes with rabbit phospho-LIMK1 (thr508)/LIMK2 (thr505) antibody (#3841) (Cell Signaling Technology, Danvers, MA, USA), rabbit anti LIMK1 (GTX10561-50) (GeneTex, Irvine, CA, USA), rabbit anti cofilin (D59) (Cell Signaling Technology, Danvers, MA, USA), mouse anti p-cofilin 1 (sc-365882), mouse monoclonal anti calponin 1/2/3 (sc-136987), mouse monoclonal anti pan-cytokeratin (sc-8018), mouse monoclonal anti β-actin antibody (sc-47778) (Santa Cruz Biotechnology, Santa Cruz, CA, USA), or mouse monoclonal anti PSA (sc-7316) at 4 °C

overnight. All primary antibodies were diluted in PBS containing 0.1 % Tween 20 (PBS-T) and 5 % milk powder.

- Washing the membranes with PBS-T three times every five minutes.
- Incubation with the secondary biotinylated horse anti mouse or horse anti goat IgG (BA-1000, BA-2000, BA-9500) (Vector Laboratories, Burlingame, CA, USA), then application of avidin and biotinylated HRP from the "Vectastain ABC kit" (Vector Laboratories, Burlingame, CA, USA), both were diluted 1:200 in PBS. Incubation lasted for 1 h at room temperature.
- Washing the membranes with PBS-T three times every five minutes.
- Washing the membranes with PBS once.
- Developing the blots with enhanced chemiluminescence (ECL) using ECL Hyperfilm (GE Healthcare, Germany).

3.5 Fluorescence staining

- Sample preparation: human prostate tissues were embedded in optimal cutting temperature (OCT) compound, and snap-frozen in liquid nitrogen and stored at -80 °C until needed.
- Cuting the prostate specimens into sections (8 µm thick) using a cryostat and then mounted on Superfrost® microscope slides (Thermo Fisher, Waltham, MA, USA). (Slides should be warmed to room temperature before staing)
- Washing the slides with PBS.
- Post-fixing the sections using methanol at -20 °C and blocked in 1 % bovine serum for 30 min.
- Incubation of the sections with primary antibodies for 1 h at room temperature. Primary antibodies were used in different sections according to the target proteins detections and listed as followed: rabbit anti LIMK1 (GTX10561-50) (GeneTex, Irvine, CA, USA), rabbit anti p-cofilin (serine

3) (77G2) (Cell Signaling Technology, Danvers, MA, USA), rabbit anti cofilin (D59) (Cell Signaling Technology, Danvers, MA, USA), mouse anti pan-cytokeratin (sc-8018), or mouse anti calponin 1/2/3 (sc-136987) (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Control groups were stained without any primary antibody.

- Washing the sections with PBST for three time every five minutes.
- Incubation of the sections with biotinylated secondary antibodies for 30 minutes at room temperature. Secondary antibodies were used in different sections according to the target proteins detections and listed as followed: Cy5-conjugated goat anti rabbit IgG (ab6564) (Abcam, Cambridge, UK), Cy3-conjugated goat anti mouse IgG (AP124C) (Millipore, Billerica, MA, USA), and fluorescein isothiocyanate- (FITC-) conjugated rabbit anti goat IgG) (AP106F) (Millipore, Billerica, MA, USA).
- Washing the sections with PBST for three time every two minutes.
- Counterstaining the Nuclei with 4', 6'-diamidino-2-phenylindole-dihydrochloride (DAPI) (Invitrogen, Camarillo, CA, USA).
- Rinsing in PBST for three time every five minutes.
- Dehydration through 95% ethanol for two min, and then 100% ethanol for twice every 3 min.
- Coverslip with anti-fade mounting medium.
- Analyzing the fluorescence with separate detectors using a confocal fluorescence microscope.

3.6 Prostate tissue tension measurements

- Krebs–Henseleit solution preparation for organ bath: put 43.5 ml KH1 and 1.62 g glucose into distilled water of 1000 ml, gas charged constantly with carbogen (95 % O_2, 5% CO_2) for 25 to 30 min, then adding 43.5 ml KH2

and gas charged for at least 5 min. KH was stored in flask and warmed and maintained at 37 °C in a thermostatic container.

- Pretension: chambers of organ bath were filled with KH solution of 10ml, KH solution was maintained at a temperature of 37 °C and carbogen (95 % O_2, 5% CO_2) was constantly charged in the chamber. Prostate strips were cut from fresh prostate tissues in the size of approximate 6 × 3 × 3 mm and then fixed in the organ bath. All the tissues were stretched to 4.9 mN and left to obtain equilibration for 45 min, during this period, spontaneous decreases in tone could be usually observed, so tension was readjusted for three times before the tone reached a stable resting tension of 4.9 mN.
- Maximal contraction of prostate strips: 400 µl of 2 M KCl (final concentration: 80mM in chamber) was added to each chamber to induce the maximal contraction of prostate strip, after that, the chambers were washed with KH solution for three times in a total volume of 30 ml.
- Inhibitors/agonist preparation and incubation: SR7826 and LIMKi3 were dissolved in DMSO, 10 µl of 1 mM SR7826, or 10 µl of 1 mM LIMKi3 was added in chamber as experiment group, and reached the final concentration to 1 µM, respectively. 10 µl of DMSO was added in chamber as control. Both inhibitors and solvent were applied to prostate strips at least 30 min before adding agonists or electrical field stimulus (EFS).
- Agonist- and EFS- induced contractions: frequencies of EFS in this study were set as 2 Hz, 4 Hz, 8 Hz, 16 Hz, and 32 Hz. Agonists and concentrations used in this study were listed in table 5-9 as follow:

Table 5. Concentrations of noradrenaline in organ bath.

Concentration	Volume	Final concentration in organ bath
0.1 mM	10 µl	0.1 µM
0.1 mM	20 µl	0.3 µM
1 mM	7 µl	1 µM
1 mM	20 µl	3 µM
10 mM	7 µl	10 µM
10 mM	20 µl	30 µM
10 mM	70 µl	100 µM

Table 6. Concentrations of phenylephrine in organ bath.

Concentration	Volume	Final concentration in organ bath
0.1 mM	10 µl	0.1 µM
0.1 mM	20 µl	0.3 µM
1 mM	7 µl	1 µM
1 mM	20 µl	3 µM
10 mM	7 µl	10 µM
10 mM	20 µl	30 µM
10 mM	70 µl	100 µM

Table 7. Concentrations of methoxamine in organ bath.

Concentration	Volume	Final concentration in organ bath
0.1 mM	10 µl	0.1 µM
0.1 mM	20 µl	0.3 µM
1 mM	7 µl	1 µM
1 mM	20 µl	3 µM
10 mM	7 µl	10 µM
10 mM	20 µl	30 µM
10 mM	70 µl	100 µM

Table 8. Concentrations of U46619 in organ bath.

Concentration	Volume	Final concentration in organ bath
0.1 mM	10 µl	0.1 µM
0.1 mM	20 µl	0.3 µM
1 mM	7 µl	1 µM
1 mM	20 µl	3 µM
10 mM	7 µl	10 µM
10 mM	20 µl	30 µM

Table 9. Concentrations of endothelin-1 in organ bath.

Concentration	Volume	Final concentration in organ bath
0.4 mM	2.5 µl	0.1 µM
0.4 mM	5 µl	0.3 µM
0.4 mM	17.5 µl	1 µM
0.4 mM	50 µl	3 µM

- Agonist- and EFS- induced contractions calculation: KCl- induced prostate smooth muscle contraction was set as 100%, agonists- and EFS- induced contractions were expressed as % of KCl- induced contractions.

3.7 Phalloidin staining

- Cells were digested by tryptase, then transferred and plated on Lab-Tek chamber slides (Thermo Fisher, Waltham, MA, USA) at 37 °C in a humidified atmosphere with 5% CO_2 for culture until reaching a degree of fusion to 80%.
- Cells were applied with SR7826 or LIMKi3 in the concentrations of 5 μM and 10 μM, respectively for 24 h. Control group was applied with solvent DMSO.
- Washed the cells with PBS (pre-warmed to 37°C by water-bath) for twice.
- Cells were fixed by 3.7% paraformaldehyde solution at room temperature for 5 – 10 min, and washed again PBS (pre-warmed to 37°C by water-bath).
- Cells were stained with 100 μM fluorescein isothiocyanate- (FITC-) labeled phalloidin (Sigma-Aldrich, Munich, Germany) at room temperature for 40 min.
- Washed the cells with PBS for three times to remove the unbound phallloidin conjugate.
- Removed residual water in the cells and added antifade mounting medium to the labeled cells.
- Used a laser scanning microscope (Leica SP2, Wetzlar, Germany) to analyze the labeled cells.

3.8 Viability assay

- Dispension of 100 µl of cell suspension (20,000 cells/ well) in a 96-well plate and incubated in a humidified incubator (5% CO_2, 37°C) for 24 h.
- Application of inhibitors SR7826 or LIMKi3 into wells to reach concentrations to 1 µM or 5 µM, respectively. Added solvent DMSO as control.
- Incubation of the cells in the incubator for different length of time: 24 h, 48 h or 72 h.
- Adding 10 µl of [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium monosodium salt (WST-8) from Cell Counting Kit-8 (CCK-8) to each well of the plate at the end of each time point (24 h, 48 h or 72 h). Performed the separate control experiments for each time point. (Avoiding introduce bubbles to the wells in this procedure)
- Incubation of the plate for 2 h in the incubator.
- Measuring the absorbance at 450 nm performed with a microplate reader.

3.9 Statistical analysis

Data in this study were presented as means ± standard error of the mean (SEM) with the indicated number of each experiment. Both E_{max} and EC_{50} values were calculated by curve fitting of single experiment using GraphPad Prism 6 (Statcon, Witzenhausen, Germany). Multivariate analysis of variance (ANOVA) and two-way ANOVA were used to calculate paired or unpaired observations performed with SPSS® 20.0 (IBM SPSS Statistics. Armonk, New York: IBM Corporation.). P values <0.05 were considered to be statistically significant.

4 Results

4.1 Detection of LIMK and cofilin in human prostate tissues

4.1.1 Detection of target proteins by Western blotting

Prostate tissues from eight patients were subjected to Western blotting for detection of LIMK and cofilin. Western blot analysis of prostate tissues using an antibody against LIMK1 revealed bands at 72 kDa which hits the expected molecular weight of LIMK1. These target bands could be observed in each sample included in this study, while the intensity of bands obtained from each sample differed (Figure 3). Western blot analysis of prostate tissues using an antibody against cofilin 1 revealed bands at 18.5 kDa which hits the expected molecular weight of cofilin 1. These target bands could be observed in each sample included in this study, and the intensity of bands obtained from each sample revealed similar (Figure 3).

Western blot analysis of calponin and pan-cytokeratin were used as markers for smooth muscle and epithelial glandular cells in included samples, respectively. Bands of calponin and pan-cytokeratin were observed in the molecular weight of 34 kDa and 37-55 kDa, respectively, despite various intensity of bands were obtained from different samples. PSA was also detected in most of the included samples in the expected molecular weight of 29 kDa, and obvious variation in bands intensity from different patients were also observed (Figure 3).

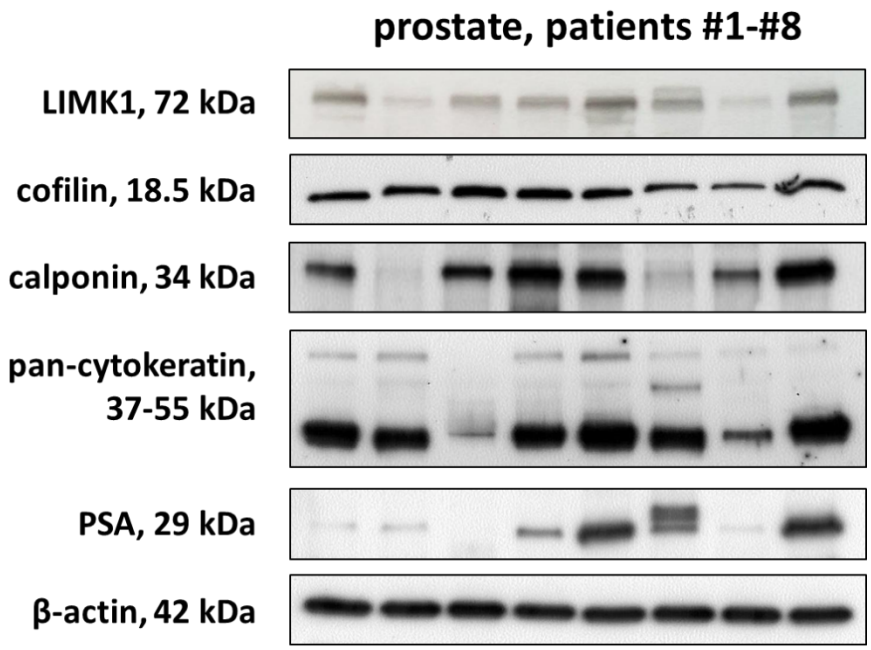


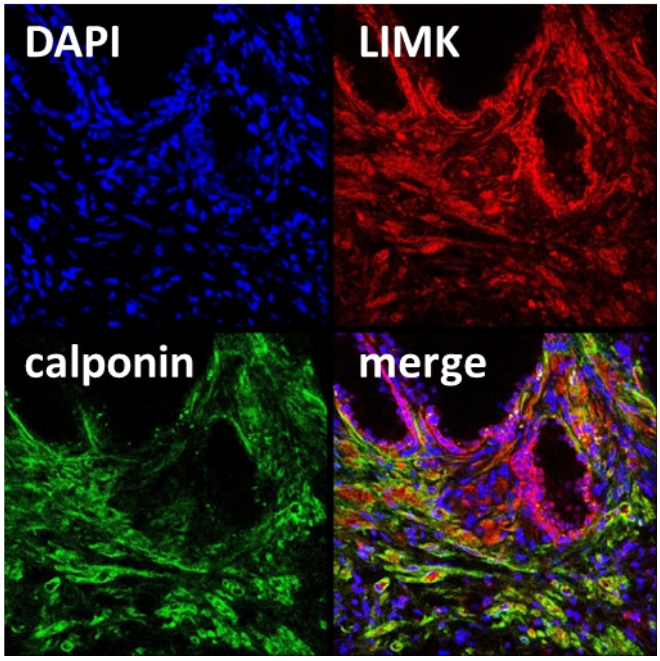
Figure 3: Detection of LIMK and cofilin in human prostate tissues. Prostate tissues for Western blot analysis were from n= 8 patients. Bands of LIMK1 and cofilin were observed in the expected molecular weight of 72 kDa and 18.5 kDa from all the included samples. Bands of calponin in molecular weight 34 kDa were observed and served as a marker for smooth muscle cells. Bands of pan-cytokeratin in molecular weight 37-55 kDa were observed and served as a marker for endothelial cells (glands). Bands of prostate-specific antigen (PSA) in were in molecular weight 29 kDa were observed and served as a marker for different degrees of benign prostate hyperplasia.

4.1.2 Detection of target proteins by fluorescence staining

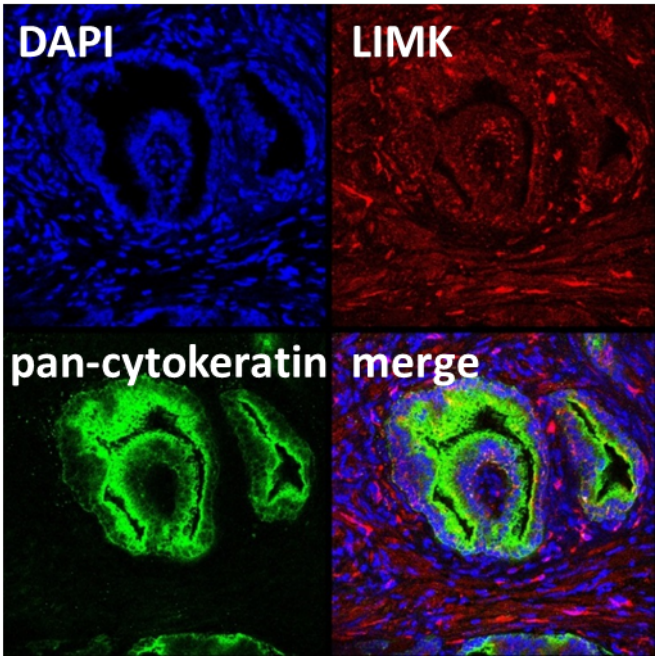
Fluorescence stainings of prostate sections were performed using anti-LIMK1, anti-calponin, anti-phospho-calponin, calponin, or pan-cytokeratin antibodies. As shown in Figure 4A, with antibodies against LIMK1, calponin and pan-cytokeratin, colocalization with immunoreactivity for calponin and pan-cytokeratin indicated that LIMK1 located in smooth muscle (Figure 4A), and limitedly extent to glandular epithelium (Figure 4A).

With an antibody raised against cofilin 1, colocalization with immunoreactivity suggested that cofilin 1 located in the stroma (Figure 4B), and to lesser extent in the glandular epithelium (Figure 4B2). Also, colocalization with immunoreactivity for calponin indicated localization of the cofilin immunoreactivity in smooth muscle cells (Figure 4B). With an antibody raised against phospho-calponin, colocalization with immunoreactivity suggested that phospho-calponin located in the stroma (Figure 4A1), and to lesser extent in the glandular epithelium (Figure 4C2).

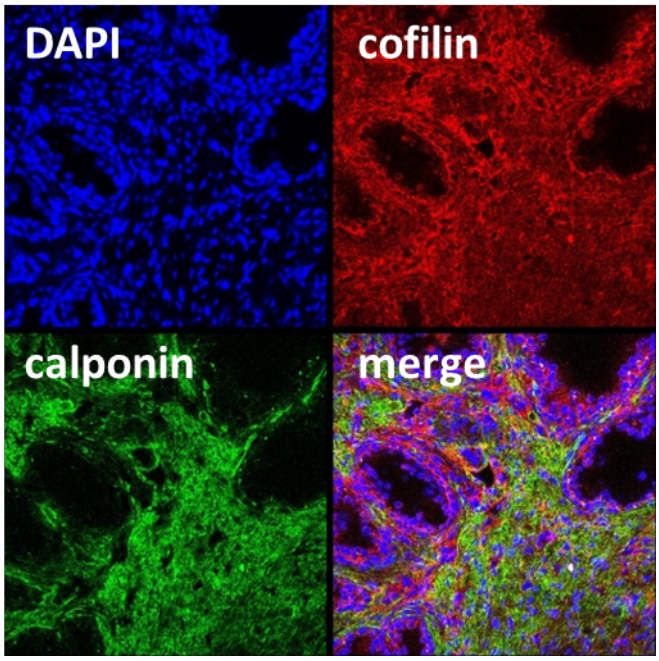
A1



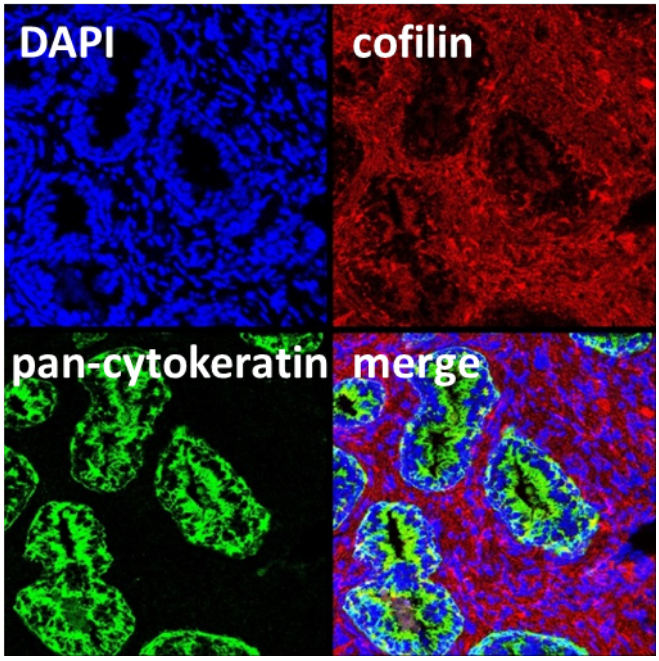
A2


DAPI
LIMK
pan-cytokeratin
merge

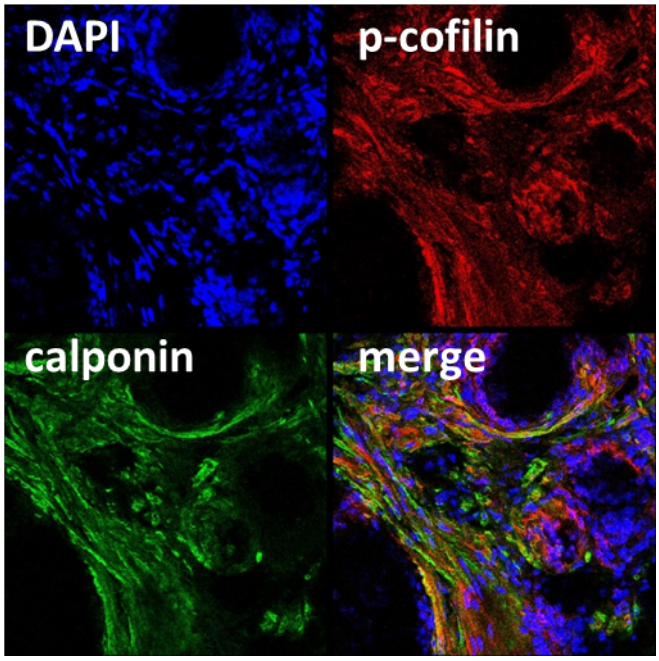
B1


DAPI
cofilin
calponin
merge

B2


DAPI
cofilin
pan-cytokeratin
merge

C1


DAPI
p-cofilin
calponin
merge

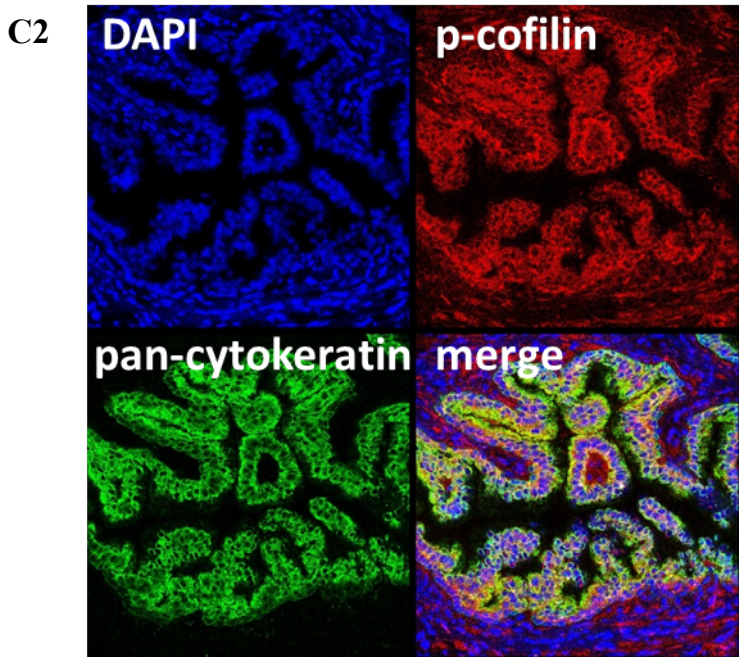


Figure 4: Immunofluorescence staining of human prostate tissues. Sections were double labelled with antibodies for LIMK1 (A), cofilin (B), or phospho-cofilin (p-cofilin) (C), together with antibodies for calponin (marker for smooth muscle cells) or pan-cytokeratin (marker for glandular epithelial cells). Yellow color in merged pictures indicates colocalization of targets. Shown are representative stainings from series with tissues from n=5 patients for each combination.

4.1.3 Detection of LIMK mRNA by RT-PCR in prostate tissue and WPMY-1 cells

Expression levels of LIMK1 and LIMK2 in prostate tissue and WPMY-1 cells were detected by RT-PCR. Prostate tissues were from seven patients, and cells from four independent experiments. Expression levels of LIMK1 and LIMK2 in each prostate sample revealed different. Remarkable higher expression levels of both LIMK1 and LIMK2 were detected in prostate tissue comparing to WPMY-1 cells (Figure 5).

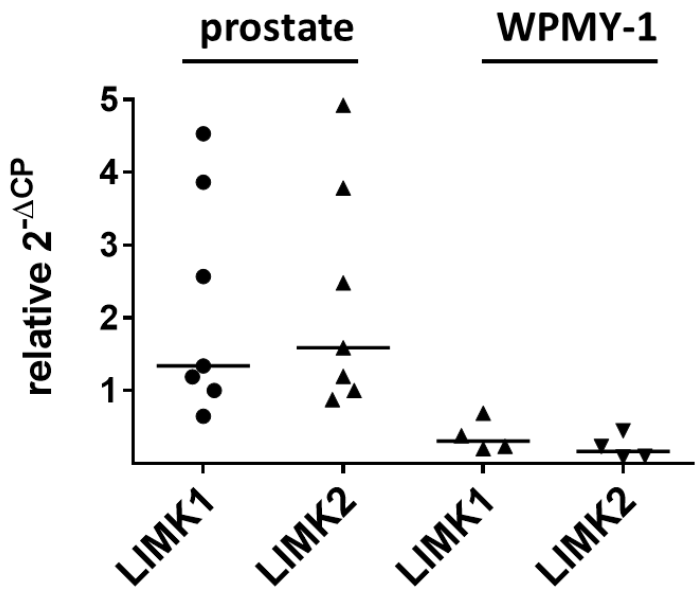


Figure 5: Detection of LIMK in human prostate tissues and WPMY-1 cells. Samples of prostate tissues and WPMY-1 cells were subjected to RT-PCR (tissues from n=7 patients, and cells from n=4 independent experiments). Data were ΔΔCP values (2^-(Ct_{target}-Ct_{GAPDH}), normalized to each other) and median values (bar).

4.2 Effects of SR7826 and LIMKi3 on noradrenaline-induced contractions

Noradrenaline (0.1- 100 µM) induced concentration-dependent contractions of prostate strips, which were inhibited by SR7826 (1 µM) and LIMKi3 (1 µM). Multivariate analysis showed that inhibiting effects of SR7826 were significant at 30 µM (p=0.046) and 100 µM (p=0.045) noradrenaline, and that inhibiting effects of LIMKi3 were significant at 30 µM (p=0.029) noradrenaline (Figure 6). Comparisons between inhibitor and control groups conducted by two-way ANOVA indicated significant inhibition of noradrenaline-induced contractions by SR7826 (p=0.006) and LIMKi3 (p=0.002).

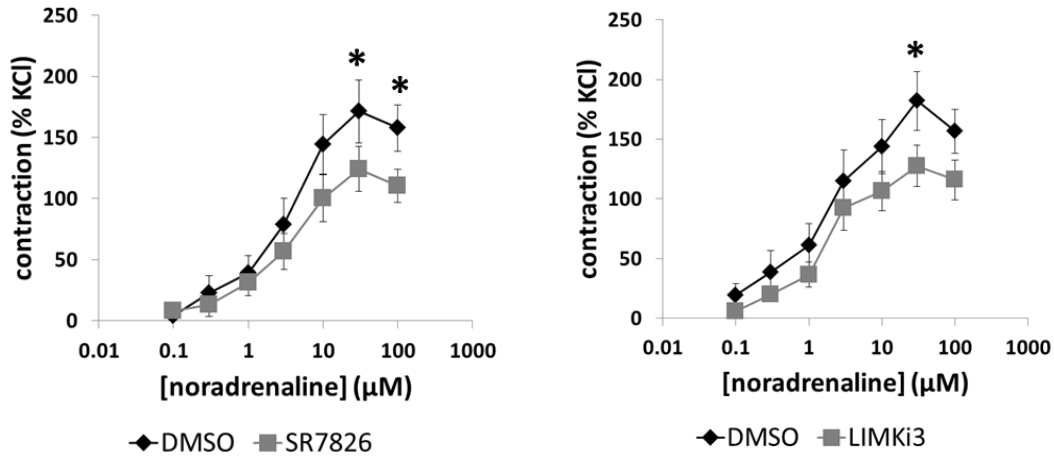


Figure 6: Effects of SR7826 and LIMKi3 on noradrenaline-induced contractions of human prostate strips. Contractions of human prostate strips were induced by noradrenaline in cumulative concentrations in an organ bath. Effects of SR7826 or LIMKi3 on contractions were compared with DMSO (control) in separate sets of experiments. To eliminate heterogeneities due to different degrees of BPH and smooth muscle contents, tensions were expressed as % of contraction induced by 80 mM KCl, which was assessed before application of inhibitors and solvent. Data are shown as means ± SEM from series with tissues from n=13 for SR7826, and n=9 for LMKi3, in which samples from every patient were assigned in both inhibitor and control groups (* $P< 0.05$ for control vs. inhibitor).

4.3 Effects of SR7826 and LIMKi3 on phenylephrine-induced contractions

Phenylephrine (0.1- 100µM) induced concentration-dependent contractions of prostate strips, which were inhibited by SR7826 (1 µM) and LIMKi3 (1 µM). Multivariate analysis showed that inhibiting effects of SR7826 were significant at 10 µM ($p=0.017$), 30 µM ($p=0.014$) and 100 µM ($p=0.015$) phenylephrine, and that the inhibiting effects of LIMKi3 were significant at 10 µM ($p=0.004$) and 30 µM ($p=0.016$) phenylephrine (Figure 7). Comparisons between inhibitor

and control groups conducted by two-way ANOVA indicated significant inhibition of phenylephrine-induced contractions by SR7826 ($p<<0.001$) and LIMKi3 ($p<<0.001$).

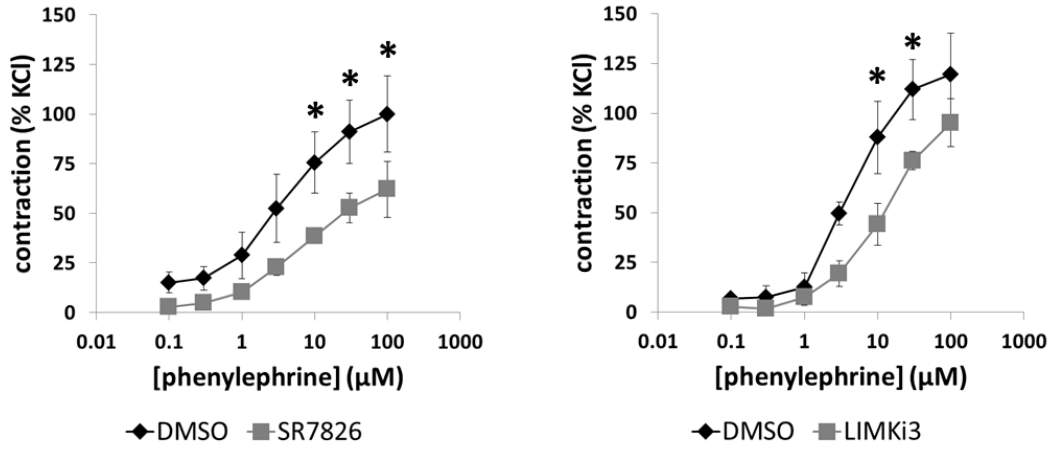


Figure 7: Effects of SR7826 and LIMKi3 on phenylephrine-induced contractions of human prostate strips. Contractions of human prostate strips were induced by phenylephrine in cumulative concentrations in an organ bath. Effects of SR7826 or LIMKi3 on contractions were compared with DMSO (control) in separate sets of experiments. To eliminate heterogeneities due to different degrees of BPH and smooth muscle contents, tensions were expressed as % of contraction induced by 80 mM KCl, which was assessed before application of inhibitors and solvent. Data were showed as means ± SEM from series with tissues from n=9 for SR7826, and n=5 for LMKi3, in which samples from every patient were assigned in both inhibitor and control groups (* $P< 0.05$ for control vs. inhibitor).

4.4 Effects of SR7826 and LIMKi3 on methoxamine-induced contractions

Methoxamine (0.1- 100µM) induced concentration-dependent contractions of prostate strips, which were inhibited by SR7826 (1 µM) and LIMKi3 (1 µM).

Multivariate analysis showed that inhibiting effects of SR7826 were significant at 30 µM (p=0.018) and 100 µM (p=0.021) methoxamine, and that the inhibiting effects of LIMKi3 were significant at 10 µM (p=0.006), 30 µM (p=0.012) and 100 µM (p=0.032) methoxamine (Figure 8). Comparisons between inhibitor and control groups conducted by two-way ANOVA indicated significant inhibition of methoxamine-induced contractions by SR7826 (p=0.008) and LIMKi3 (p=0.001).

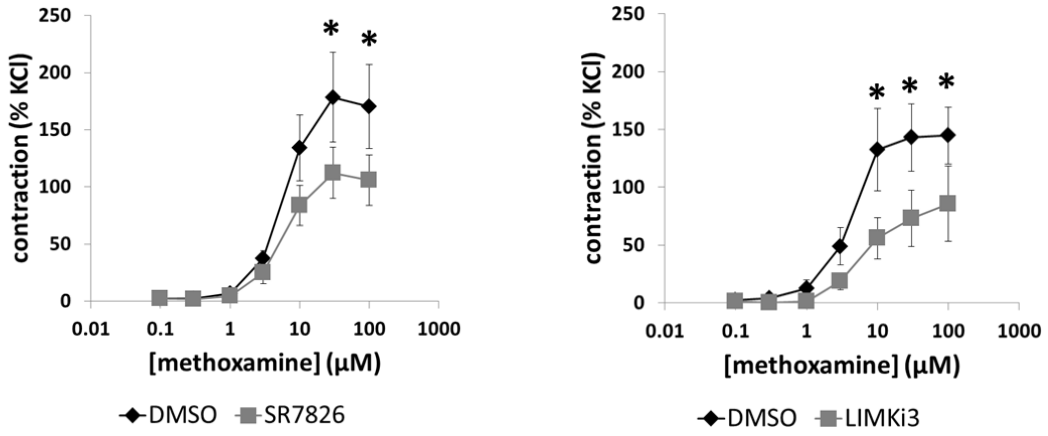


Figure 8: Effects of SR7826 and LIMKi3 on methoxamine- induced contractions of human prostate strips. Contractions of human prostate strips were induced by methoxamine in cumulative concentrations in an organ bath. Effects of SR7826 or LIMKi3 on contractions were compared with DMSO (control) in separate sets of experiments. To eliminate heterogeneities due to different degrees of BPH and smooth muscle contents, tensions were expressed as % of contraction induced by 80 mM KCl, which was assessed before application of inhibitors and solvent. Data were showed as means ± SEM from series with tissues from n=6 for SR7826, and n=5 for LMKi3, in which samples from every patient were assigned in both inhibitor and control groups (* P< 0.05 for control vs. inhibitor).

4.5 Effects of SR7826 and LIMKi3 on endothelin-1-induced contractions

Endothelin-1 (0.1- 3 μM) induced concentration-dependent contractions of prostate strips. Two preliminary series (n=3 prostates/group for both SR7826 and LIMKi3) did not indicate the possibilities that SR7826 and LIMKi3 may have inhibiting effects on endothelin-1-induced contractions (Figure 9), so that these exploratory series were halted after three cases.

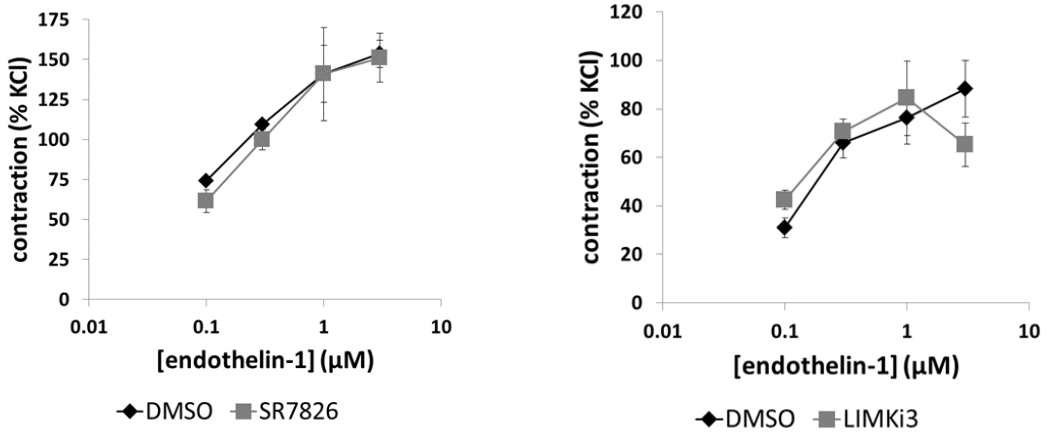


Figure 9: Effects of SR7826 and LIMKi3 on endothelin-1- induced contractions of human prostate strips. Contractions of human prostate strips were induced by endothelin-1 in cumulative concentrations in an organ bath. Effects of SR7826 or LIMKi3 on inhibiting contractions were compared with DMSO (control) in separate sets of experiments. To eliminate heterogeneities due to different degrees of BPH and smooth muscle contents, tensions were expressed as % of contraction induced by 80 mM KCl, which was assessed before application of inhibitors and solvent. Data were showed as means ± SEM from series with tissues from n=3 for both SR7826 and LMKi3, in which samples from every patient were assigned in both inhibitor and control groups.

4.6 Effects of SR7826 and LIMKi3 on U46619-induced contractions

U46619 (0.1- 30μM) induced concentration-dependent contractions of prostate strips, which were inhibited by SR7826 (1 μM) and LIMKi3 (1 μM). Multivariate analysis showed that inhibiting effects of SR7826 were significant at 1 μM ($p=0.041$), 3 μM ($p=0.028$) and 10 μM ($p=0.024$) U46619, and that the inhibiting effects of LIMKi3 were significant at 1 μM ($p=0.04$), 3μM ($p=0.021$), 10 μM ($p=0.007$) and 30 μM ($p=0.011$) U46619 (Figure 10). Comparisons between inhibitor and control groups conducted by two-way ANOVA indicated significant inhibition of U46619-induced contractions by SR7826 ($P<0.001$) and LIMKi3 ($P<0.001$).

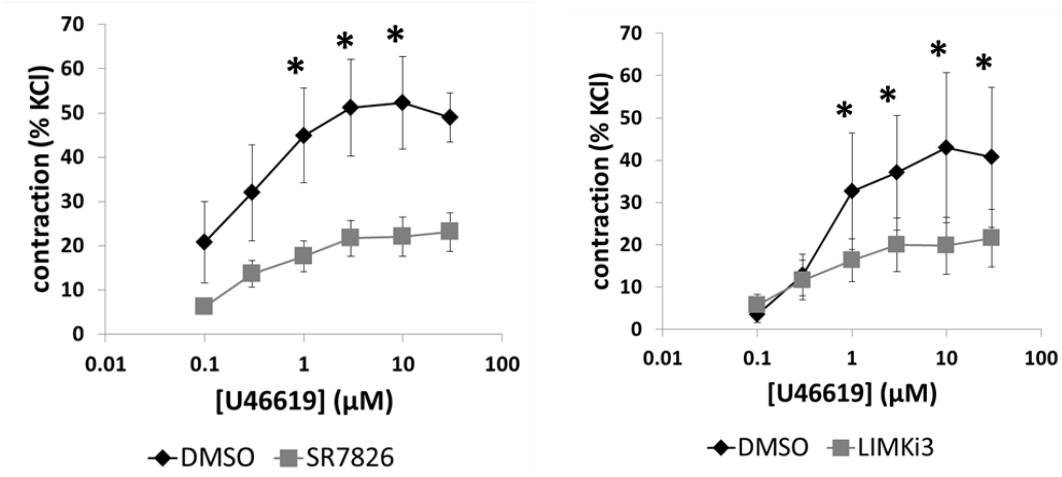


Figure 10: Effects of SR7826 and LIMKi3 on U46619- induced contractions of human prostate strips. Contractions of human prostate strips were induced by U46619 in cumulative concentrations in an organ bath. Effects of SR7826 or LIMKi3 on contractions were compared with DMSO (control) in separate sets of experiments. To eliminate heterogeneities due to different degrees of BPH and smooth muscle contents, tensions were expressed as % of contraction induced by 80 mM KCl, which was assessed before application of inhibitors and solvent. Data were showed as means ± SEM from series with tissues from n=5 for both SR7826 and LMKi3, in which samples from every patient were assigned in both inhibitor and control groups (* P< 0.05 for control vs. inhibitor).

4.7 Effect of SR7826 and LIMKi3 on EFS-induced contractions

EFS (2-32 Hz) induced frequency-dependent contractions of prostate strips, which were inhibited by SR7826 (1 µM) and LIMKi3 (1 µM). Multivariate analysis showed that inhibiting effects of both SR7826 and LIMKi3 were significant at 16 Hz (p=0.013 for SR7826, p=0.04 for LIMKi3) and 32 Hz (p=0.002 for SR7826, p=0.008 for LIMKi3) (Figure 11). Comparisons between inhibitor and control groups conducted by two-way ANOVA indicated significant inhibition of EFS-induced contractions by SR7826 (p=0.002) and

LIMKi3 (p=0.018).

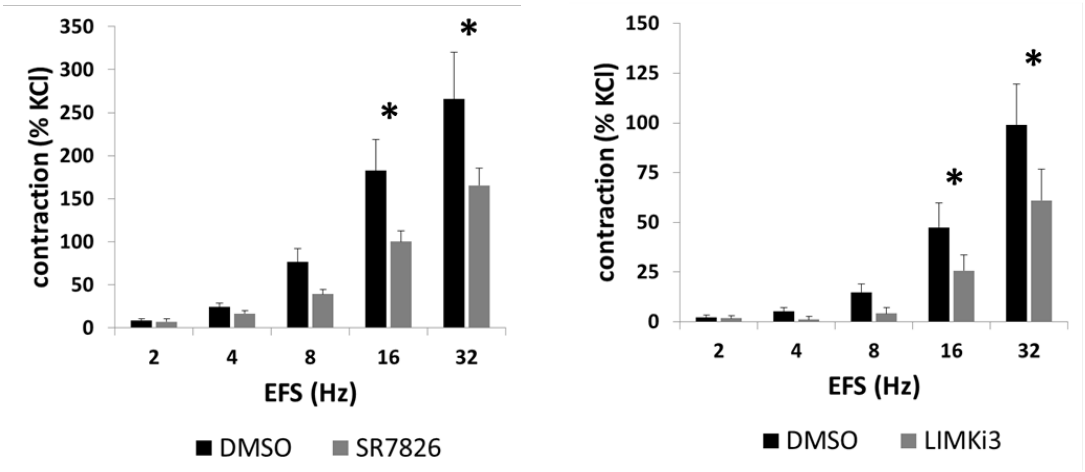


Figure 11: Effects of SR7826 and LIMKi3 on EFS- induced contractions of human prostate strips. Contractions of human prostate strips were induced by EFS in different frequencies in an organ bath. Effects of SR7826 or LIMKi3 on contractions were compared with DMSO (control) in separate sets of experiments. To eliminate heterogeneities due to different degrees of BPH and smooth muscle contents, tensions were expressed as % of contraction induced by 80 mM KCl, which was assessed before application of inhibitors and solvent. Data were showed as means ± SEM from series with tissues from n=5 for both SR7826 and LMKi3, in which samples from every patient were assigned in both inhibitor and control groups (* P< 0.05 for control vs. inhibitor).

4.8 Effect of SR7826 and LIMKi3 on cofilin phosphorylation

Prostate tissues were incubated with either SR7826 or LIMKi3 (both 1 μM) for 0.5 h. Subsequently, serine-3-phosphorylated cofilin was detected by Western blotting using a site- and phospho-specific antibody. As shown in Figure 12, the content of phospho-cofilin in prostate tissues was reduced by SR7826 and LIMKi3. SR7826 reduced the phospho-cofilin content by 59 ±15 % compared

with control, while LIMKi3 reduced the content of phospho-cofilin by 56 ±13 %. These two inhibitors seemed to have no effect on total cofilin which revealed unchanged contents after incubation, also, the content of β-actin remained unchanged.

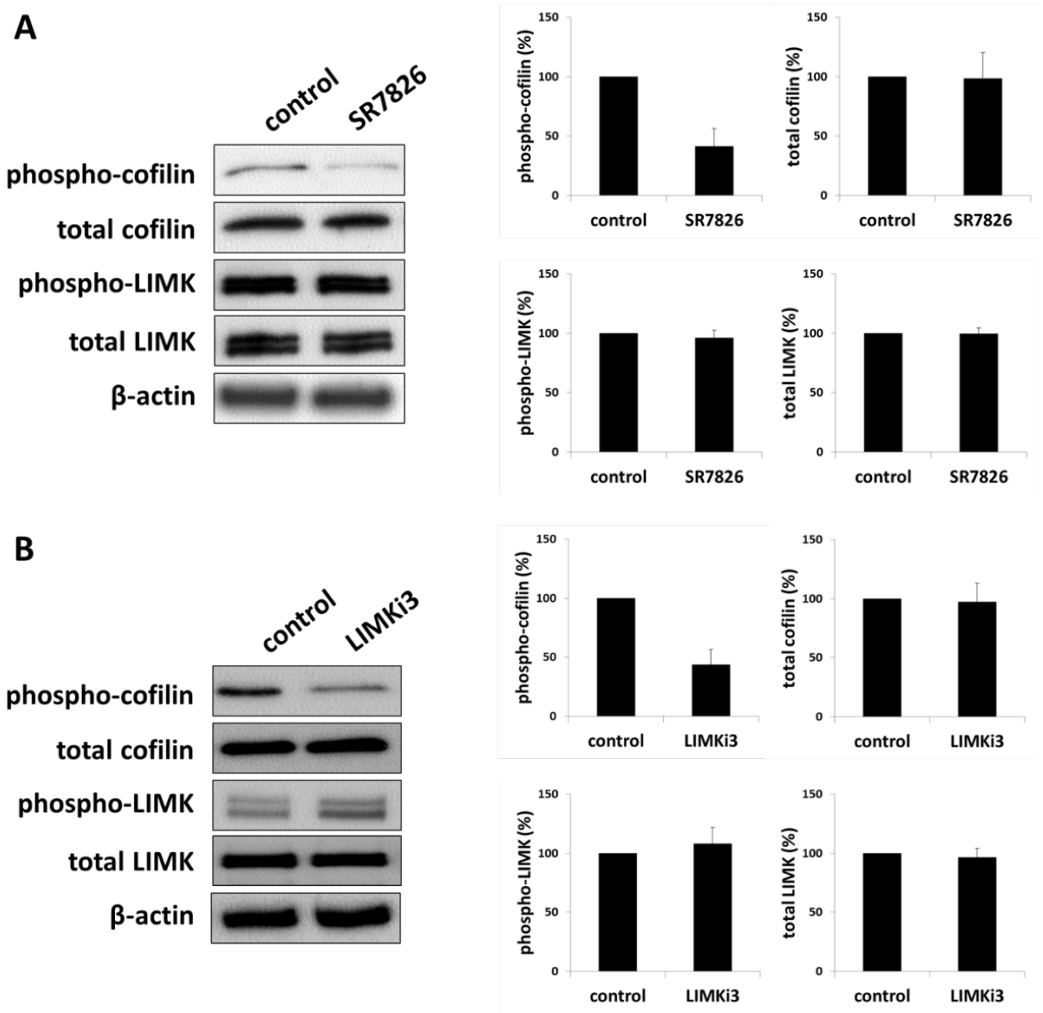
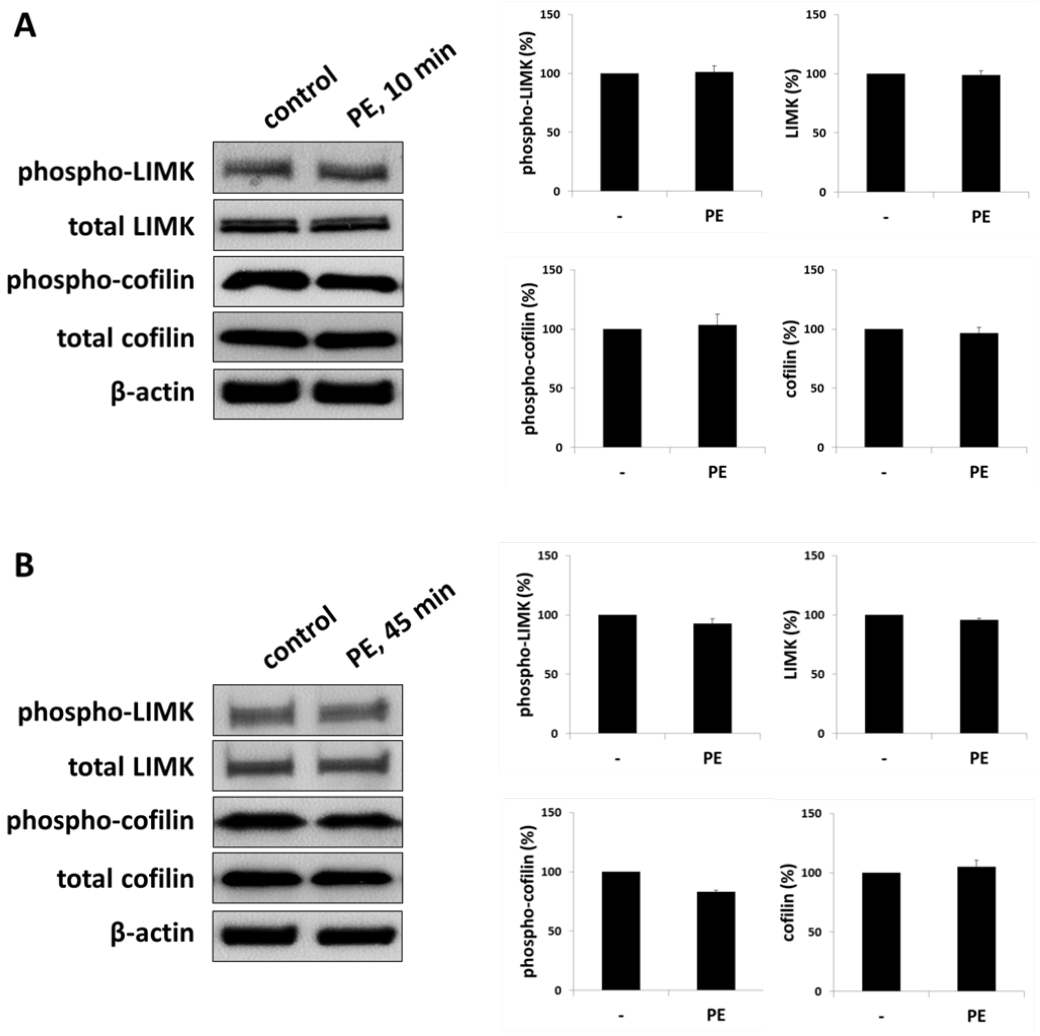


Figure 12: Effects of SR7826 and LIMKi3 on cofilin phosphorylation in human prostate tissues. Prostate tissues were incubated with SR7826 (1 μM) or DMSO (control) **(A)**, or with LIMKi3 (1 μM) or DMSO (control) **(B)** in separate series of experiments. The phosphorylation state of cofilin was then semiquantitatively compared between both inhibitor and control groups performed with Western blotting using a site- and phospho-specific cofilin antibody. Value for each sample was normalized to the mean of each corresponding control group. Data were shown as representative blots, and means ± SEM from series with tissues from patients n=7 (SR7826) or n=6 patients (LIMKi3).

4.9 Effect of phenylephrine and U46619 on LIMK and cofilin phosphorylation

Prostate tissues were incubated with different contractile agonists, and contents of phospho-LIMK, LIMK, phospho-cofilin, cofilin, and β-actin were then assessed by Western blotting. As shown in Figure 13, after incubation with phenylephrine (30 μM) for 10 (Figure 13A) or 45 min (Figure 13B), or with U46619 (30 μM) for 1 h (Figure 13C), the content of phospho-LIMK, LIMK, phospho-cofilin, cofilin, or β-actin in prostate tissues remained unchanged.



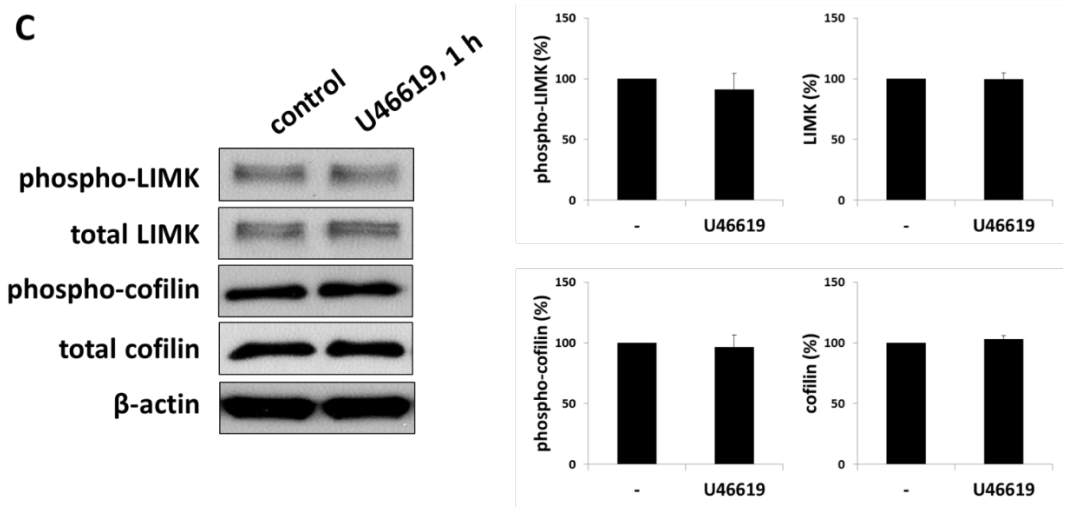


Figure 13: Effects of phenylephrine and U46619 on LIMK and cofilin phosphorylation in human prostate tissues. In each experiment, prostate tissues were incubated with phenylephrine ("PE") (100 µM) for 10 min (A), or 45 min (B), or with U46619 (30 µM) for 1 h (C), or with solvent (water for PE, ethanol for U46619). Subsequently, Western blot analyses performed with site- and phospho-specific antibodies were used for semiquantitative comparison of the phosphorylation states of LIMK and cofilin between agonist and control groups. The values of total LIMK and cofilin in each sample were also detected to normalize to the mean of the corresponding control group (samples without treating with agonists). Data were presented as means ± SEM from series with tissues from patients n=5 (PE for 10 min), n=3 (PE for 45 min), or n=7 (U46619 for 1 h).

4.10 Effect of SR7826 and LIMKi3 on viability of WPMY-1 cells

Both inhibitors, SR7826 and LIMKi3, showed a concentration-dependent effect on viability of WPMY-1 cells. At the concentration of 1 µM (which significantly inhibited smooth muscle contraction of prostate stripes) of SR7826 and LIMKi3, only minor reduced viabilities were observed after exposure to inhibitors for 24h (control vs. SR7826 and LIMKi3= 1.197: 1.133

and 1.103), 48h (1.246: 1.127 and 1.049) or 72h (1.357: 1.193 and 1.082) (Figure 14). At the concentration of 5 µM, both inhibitors showed markedly reduced effects on cells viability after 24h (control vs. SR7826 and LIMKi3= 1.197: 1.027 and 0.951), 48h (1.246: 1.004 and 0.8264), or 72h (1.357: 1.054 and 0.770) of exposure. Minor altered viabilities of cells in each separated period (24, 48, or 72h) were observed after exposure to the same concentration (1 µM or 5 µM) of SR7826 or LIMKi3, which failed to show a time-dependent effect on viability of WPMY-1 cells.

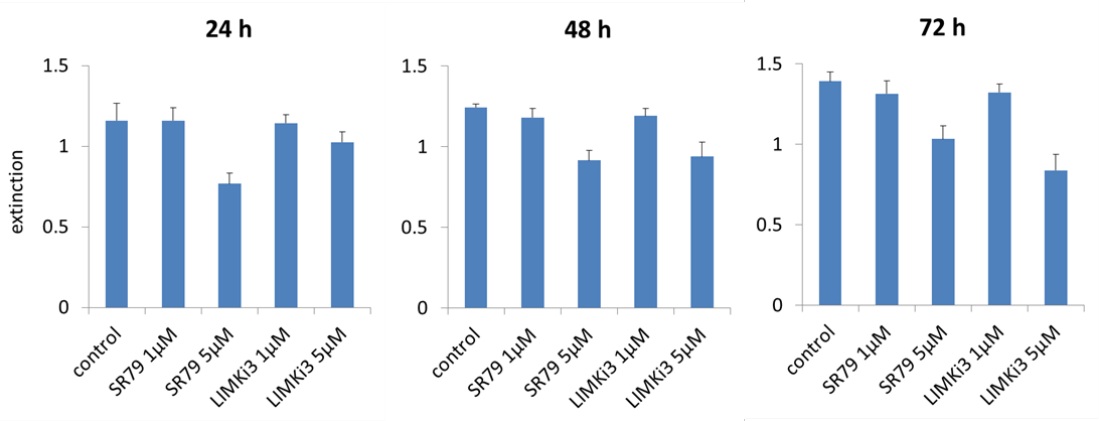


Figure 14: Concentration-dependent effects of SR7826 and LIMKi3 on WPMY-1 cells. WPMY-1 cells were exposed to SR7826 or LIMKi3 in concentrations of 1 µM or 5 µM for 24, 48, or 72h, respectively. Cells without inhibitors under the same conditions were treated as controls. Data were shown as means ± standard deviation (SD) from n=5 independent experiments.

4.11 Effects of SR7826 and LIMKi3 on actin organization of WPMY-1 cells

Polymerized actin in WPMY-1 cells was visualized by phalloidin staining. In control groups, where cells were treated with solvent DMSO, actin was observed to be organized to thin and long filaments, and filamentous

protrusions of different cells overlapped each other (Figure 15). Exposure to SR7826 or LIMKi3 for 24 h revealed concentration-dependent effects on actin filaments. After exposure with 5 µM SR7826, filaments length was reduced in some cells. Cells which were still visible revealed similar actin organization to cells in controls, but these were of lesser amount (Figure 15A). Extensive breakdown of actin organization was observed after exposure with 10 µM SR7826, so that phalloidin-stained actin was invisible in most cells and only very short filaments remained in very few cells (Figure 15B). The concentrations of 5 and 10 µM of LIMKi3 showed similar, concentration-dependent effects on extensive breakdown of actin organization: actin filaments partly disappeared after exposure to 5 µM of LIMKi3 after 24 h, and after exposure to 10 µM of LIMKi3 for 24 h, remaing phalloidin-stained actin was found to be shorter, and completely restricted to the rim of some nuclei (Figure 15B).

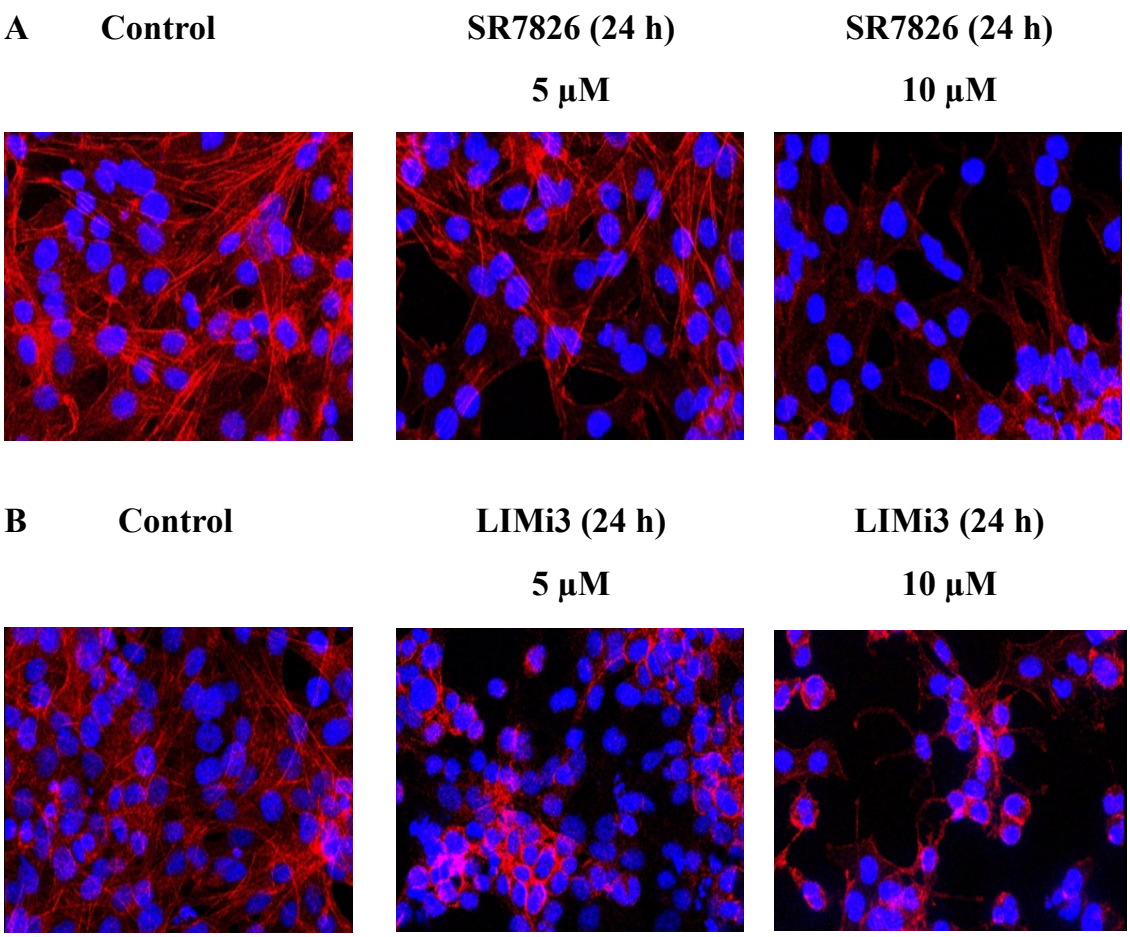


Figure 15: Effects of SR7826 and LIMKi3 on WPMY-1 cells. WPMY-1 cells were exposed to SR7826 or LIMKi3 in concentrations of 5 µM or 10 µM for 24 h. WPMY-1 cells treated with slovent DMSO under the same conditions were set as controls. Both SR7826 (A) and LIMKi3 (B) showed concentration-dependent effects on actin organization of WPMY-1 cells. Shown are representative pictures from n=3 independent experiments in both (A) and (B).

5 Discussion

The findings in this thesis suggest that LIMK inhibitors may inhibit prostate smooth muscle contraction in vitro. To the best of my knowledge, this is the first study to explore the effects of small molecule LIMK inhibitors on regulation of smooth muscle contraction in the prostate or even in any organ.

Prostate tissues used in this study were obtained from patients undergoing radical prostatectomy due to prostate cancer. All the samples were taken from periurethral zone, while most prostate tumors are located to the peripheral zone (Pradidarcheep et al., 2011; Shaikhibrahim et al., 2012). Samples were characterized by high variations in the content of PSA which could be detected by Western blotting (Figure 3). As PSA increases with degree of hyperplasia, this may suggest different grades of BPH in the investigated samples (Levitt and Slawin, 2007). Actually, around 80% or more patients undergoing prostatectomy accompany with BPH with different degrees (Alcaraz et al., 2009; Orsted and Bojesen, 2013). So the samples used in this study may be considered as hyperplastic, but not malignant. Prostate tissues from patients undergoing TURP may show more pronounced hyperplasia. However, tissues may be seriously traumatized or denaturation after being resected from the prostate, and the size of any available samples may be lower than that obtained from radical prostatectomy. Anyway, in most patients undergoing TURP, the periurethral zone will be mostly ablated, so that samples from this zone are not accessible any more. For these reasons, prostate tissues were obtained from patients underwent prostatectomy instead TURP.

Different content of calponin between investigated samples was detected by Western blotting. This reflects a diverging content of smooth muscle between

the samples from different prostates. Different content of pan-cytokeratin between investigated samples was also observed, probably due to divergent stromal/glandular ratio in samples, which may reflect the individual variation of tissue composition of prostates (Figure 3). Immunofluorescence staining of pan-cytokeratin and calponin showed typical architecture composed of stroma and glands but also with variation between each invested sample (Figure 4). Notably, the positive detection of pan-cytokeratin and calponin may thereby confirm that the investigated samples in this project contain normal glandular and prostate smooth muscle, which could guarantee the quality of studied samples. To correct the heterogeneity between samples, all contractions in organ bath experiments were referred to receptor- independent tensions induced by 2 M KCl.

LIMK1 and LIMK2 are encoded by separate genes which locate on chromosomes 7q11.23 and 22q12.2, respectively. Widespread distribution of LIMK1 and LIMK2 has been reported in previous studies. LIMK1 was found to be widely expressed in embryonic as well as tissues from mouse and human, with remarkably high expression in tissues from brain, lung, stomach, kidney and testis (Foletta et al., 2004). Compared to LIMK1, LIMK2 was also found in most of examined tissues, while with the exceptions of kidney glomeruli, testis and glial cells (Acevedo et al., 2006). In the present study, expressions of LIMK1 and LIMK2 in prostate tissues as well as WPMY-1 cells were confirmed performed with RT-PCR, western blotting and immunofluorescence (Figure 3 and 4).

Previous studies on LIMK in the prostate mainly focused on oncologic investigations. Overexpressed levels of LIMK1 in prostate adenocarcinomatous tissues and malignant prostate cell lines were observed, and it might be assumed that LIMK were essential for the invasive property and the growth of prostate cancer cells (Davila et al., 2003). In contrast, suppression of upstream

regulators in the LIMK-associated signaling pathways caused downregulation of LIMK, which thereby inhibited proliferation of prostate cancer in vitro (Cai et al., 2015; Zhu et al., 2015). Also, downregulation of LIMK inhibited by LIMK selective inhibitors has been demonstrated to reduce motility and proliferation of prostate cancer cells (Mardilovich et al., 2015).

LIMK are homologous serine/threonine kinases which may be activated by Rho/ROCK, cAMP/PKA, Rac/PAK, and Cdc42/MRCK signaling pathways (Bernard, 2007). LIMK phosphorylate cofilin proteins on serine 3 and thereby act as regulators of actin-myosin cytoskeletal dynamics, which includes actin polymerization, filament assemble, and stress fiber formation in a number of various cell types. (Hennenberg et al., 2008; Hennenberg et al., 2014b, a; San Martin et al., 2008; Scott and Olson, 2007). Previous studies on relation between LIMK and smooth muscle cells or tissues were quite few, and most of them were mainly focusing on inhibiting the upstream modulator of LIMK-associated signaling pathways which caused change in LIMK expression, but not directly upregulating or downregulating the expression of LIMK itself. Thus, direct evidence from functional studies for an involvement of LIMK in smooth muscle contraction, e. g. from application of small molecule inhibitors in organ bath studies is still absent. After treating with ROCKII inhibitor, attenuated phosphorylated LIMK1/2 and phosphorylated cofilin were observed in vascular smooth muscle cells, which thereby reduced the vascular structural remodeling (Al-Ghabkari et al., 2016). Force-induced RhoA activation was also reported to promote phosphorylation of LIMK and cofilin, and hence increased hypertrophic growth of the myocardium (Zhao et al., 2007). Platelet derived growth factor (PDGF) and IL- 1ß, were reported to promote proliferation and migration of smooth muscle cells and cause pulmonary hypertension via upregulating of phosphorylation of LIMK and cofilin (Bongalon et al., 2004; Wilson et al., 2015).

SR7826 and LIMKi3 are small molecule inhibitors, which inhibit LIMK1 (SR7826 and LIMKi3) and LIMK2 (LIMKi3) with high potency. In biochemical kinase assays, where inhibition was assessed using recombinant kinases in vitro, SR7826 inhibited LIMK1 with an IC_{50} value of 43 nM, while LIMKi3 inhibited LIMK1- and -2 with IC_{50} values of 7-8 nM (Ross-Macdonald et al., 2008; Yin et al., 2015). As to SR7826, comparing to the concentration used in this study (1 μM), its IC_{50} values for other kinases ranged much higher, for example, more than 5 μM for ROCK1 and ROCK2. It is widely accepted that, due to the different access to the inhibitors' targets resulting from barriers like connective tissue or membranes, IC_{50} values in biochemical assays are usually lower than EC_{50} values in organ bath experiments and in intact tissues (Hennenberg et al., 2016; Swinney, 2004; Vauquelin et al., 2002). So it may be reasonable that the concentration of 1 μM used for SR7826 and LIMKi3 in this study was comparably low, and still below IC_{50} values for non-specific kinase inhibition indicated in biochemical assays. Inhibition of LIMK2 by SR7826 has not been reported, so that it may be assumed that the inhibition of prostate contraction occurred by inhibition of LIMK1 rather than LIMK2. However, it is presently not possible to estimate whether inhibition of LIMK2 by SR7826 is in fact lacking, or whether this reflects lack of data.

LIMK can be phosphorylated at threonine 508 by upstream signaling pathways (e.g. ROCK isoforms), and thereby affect its substrate cofilin (Bernard, 2007). As observed in this study, the content of phospho-cofilin in prostate tissues was significantly reduced after being treated with SR7832 and LIMKi3, while the content of phospho-LIMK remain unchanged in these tissues. This may allow the conclusion that inhibition of prostate smooth muscle contraction was mainly caused by specific inhibitions of LIMK1 and/or LIMK2, but did not involve inhibition of ROCK isoforms. In fact, Rho kinases are activated by contractile receptors, so that LIMK would be observed here, if LIMK activation involved Rho kinase (Hennenberg et al., 2006). Moreover, it may be

concluded that threonine 508 is no substrate for LIMK autophosphorylation in the prostate, as no decreases in LIMK phosphorylation at this position were induced by SR7826 or LIMKi3.

In prostate tissues, inhibition of cofilin phosphorylation was limited to 56-59 %, what may be attributed to LIMK inhibition. As a certain degree of remaining phosphorylated cofilin was observed in the presence of LIMK inhibitors, a contribution of additional kinases to cofilin phosphorylation in prostate tissues and WPMY-1 cells appears possible.

In the organ bath experiments, we observed that small molecule LIMK inhibitors inhibited contractile responses induced by α_1-adrenergic receptor agonist which induced noradrenaline, phenylephrine, and methoxamine. The inhibitions ranged around 30%, which may be a little weaker than the inhibiting effect of tamsulosin, the approved clinical used α_1-adrenergic blocker (Gratzke et al., 2015; Hennenberg et al., 2017a; Oelke et al., 2013). While in the EFS-induced muscle contractions, which are assumed to be mediated by release of endogenous neurotransmitters, and further activation of postsynaptic α_1-adrenoceptors on smooth muscle cells, these two inhibitors showed inhibitions ranged around 50% that may equal or even exceed the inhibiting effect of α_1-adrenergic blocker (Buono et al., 2014; Oger et al., 2010).

Strikingly, SR7826 and LIMKi3 were also observed to inhibit agonist-induced contractions caused by the thromboxane A_2 analog U46619. It may be hypothesized that these two inhibitors could improve urodynamic parameters in vivo, which may resemble, or even exceed the effects of clinical approved used α_1-blockers. SR7826 and LIMKi3 failed to show inhibiting effects on endothelin-1-induced smooth muscle contraction after three independent experiments, so that organ bath series with endothelin-1 were discontinued for economic reasons after three experiments, because it was obvious that

inhibitions were unlikely to be expected. Endothelin-1 is one of the most important endogenous smooth muscle constrictors; it can activate DAG and Rho and further phosphorylates MLC, but not cofilin, to cause smooth muscle contraction (Bouallegue et al., 2007). It might be speculated that the different signaling pathways activated by endothelin-1 may contribute to the absence of inhibiting effects by SR7826 and LIMKi3.

It has been reported that some smooth muscle contractile agonists, which include phenylephrine, noradrenaline, endothelin-1, angiotensin II, thrombin, and 5-hydroxytryptamine (5-HT) induce LIMK activation and cofilin phosphorylation in vascular smooth muscle cells (Dai et al., 2008; Du et al., 2010). To identify whether contractile agonists could activate LIMK in prostate, their effects on LIMK and cofilin phosphorylation were tested. U46619 is a synthetic analog of prostaglandin PGH2 and acts as a thromboxane A_2 receptor agonist, while phenylephrine is a selective α_1-adrenergic receptor agonist. After being treated with phenylephrine for 10 min or 45 min, or with U46619 for 1 h, no change in the content of phospho-LIMK or phospho-cofilin was observed in these prostate tissues (Figure 13). This may suggest that that neither α1-adrenoceptors nor thromboxane A_2 receptors activate LIMK in the human prostate.

Immunofluorescence study on untreated prostate tissues suggested the detection of phospho-cofilin (Figure 13). Although this may suggest a pool of active LIMK in the prostate cells, the conclusion on whether this reflects a constitutive or inducible process could not be drawn, as other kinases, e.g. TESK1 (Toshima et al., 2001a), TESK2 (Toshima et al., 2001b), and NRK/NESK (Nakano et al., 2003), have been also reported to be able to phosphorylate cofilin. LIMK may be a crucial intracellular modulator in prostate smooth muscle contractile response, however, the role of LIMK as a signal transductor from receptors to contraction in the human prostate remained

unclear.

LIMK phosphorylate cofilin, and thereby promote actin filaments polymerization and cytoskeleton reorganization. Previous studies have reported a role of LIMKs in regulating actin organization in breast cancer cells (Bagheri-Yarmand et al., 2006), prostate cancer cells, and prostate epithelial cells (Davila et al., 2003). As observed in this study, prostate smooth muscle cells shown a breakdown of the actin cytoskeleton after being treated with SR7826 and LIMKi3 in a concentration-dependent manner, which was consistent with the previous findings from other cell types (Bagheri-Yarmand et al., 2006; Davila et al., 2003; San Martin et al., 2008; Yang et al., 1998). Since actin polymerization and cytoskeleton reorganization are considered to be a prerequisite for smooth muscle contraction, the breakdown of organization in WPMY-1 cells observed in this study (Figure 15) might thereby be responsible for reducing the smooth muscle contraction.

The concentrations of both inhibitors to induce the breakdown of actin organization in WPMY-1 cells (5 μM) were higher than those to inhibit smooth muscle contraction of prostate strips (1 μM). It may be hypothesized that the different expression levels of LIMK1 and LIMK2 between prostate tissue and WPMY-1 cells might be responsible for the different required concentrations. As detected in this study, WPMY-1 cells showed lower mRNA contents of both LIMK1 and LIMK2 compared with prostate tissues. This may explain why higher concentrations of inhibitors were required: at low content of target kinases, more inhibitor may be required to effectively address the enzymes and thereby to attain kinase inhibition. The viability assays performed in WPMY-1 cells may also confirm this hypothesis, as not any effect on WPMY-1 cells could be observed at the concentration of 1μM of SR7826 and LIMKi3. Reduced filaments length in some cells was observed at the concentrations of 5μM and 10μM, i. e. turned out to occur in a concentration-dependent manner

(Figure 15). These findings are consistent with previous studies which reported reduced viability due to LMIK deficiency or after treatment of LIMK inhibitors in different cell types, e. g. in human Schwann cells or in mesenchymal glioblastoma multiforme cells (Park et al., 2014; Petrilli et al., 2014).

Although α_1-blockers are the first line treatment for patients with LUTS/BPH, their effects are still limited, approximately up to 69% of patients might not receive benefit from α 1-blockers (Lee et al., 2015; Matsukawa et al., 2013). It was hypothesized that the non-adrenergic mediators, which cause prostate smooth muscle tone in parallel to α_1-adrenoceptors, might be responsible for the absent effects of α_1-blockers (Hennenberg et al., 2016). It would make sense that if combination treatments of inhibiting adrenergic and non-adrenergic contractions are available, higher efficacy might therefore be expected.

Taken together, this study may show an important role of LIMK in regulating smooth muscle contractile response in hyperplastic prostate. LIMK may cause prostate smooth muscle contraction of human prostate by phosphorylating cofilin and subsequent promoting actin organization in favor of the contractile state. Since LIMK could be involved in bladder outlet obstruction and urethral obstruction in BPH, it may be hypothesized that the two inhibitors studied in this research, SR7826 and LIMKi3, or even other LIMK inhibitors could have effects on improving urodynamic parameters in vivo, or releasing the symptoms of lower urinary tract. Although a number of previous studies explored the mechanisms of prostate smooth muscle contraction, and despite its crucial role in pathophysiology and therapy of LUTS in millions of patients, the understanding of prostate smooth muscle contraction is still insufficient (Hennenberg et al., 2014b, a; Hennenberg et al., 2017a). Further studies are still needed to describe its regulation and underlying mechanisms. The findings of

this study may thereby contribute new light, or a new model, to the understanding of intracellular mechanisms of prostate smooth muscle contraction. Also, considering the significant inhibiting effects of SR7826 and LIMKi3 on prostate cells and tissues, this may provide a new strategy for developing new therapeutic approaches for treatment of LUTS. In vivo study to evaluate the effects of SR7826 and LIMKi3 on improving urodynamic parameters may be warranted.

6 Conclusion

The present study suggested that LIMKs promote prostate smooth muscle contraction by phosphorylating cofilin and subsequent actin organization. LIMKs may act as important regulators in urethral obstruction and bladder outlet obstruction in patients with BPH/LUTS. The effects of LIMKs in the human prostate could be inhibited by small molecule LIMK inhibitors, SR7826 and LIMKi3, which might therefore possibly be considered as a basis for new approaches in LUTS therapy. Also, a similar role of LIMK inhibitors in regulating smooth muscle contraction in other organs might be possible.

7 Summary

LUTS refer to a group of urological symptoms that are caused by multifactorial aetiology. The prevalence of LUTS increases with age, and will thereby lead to heavy economic burden for the society. In men with benign prostate hyperplasia, increased smooth muscle tone in the prostate could result in bladder outlet obstruction and subsequent symptoms of lower urinary tract. Pharmacological treatment aiming to inhibit prostate smooth muscle contraction is considered as the option of first choice. However, the efficacy of current available treatment options is limited, thereby, improved understanding in the mechanisms of prostate smooth muscle contraction and development of novel targets for medical therapy are warranted. Previous studies have reported that LIMK (LIMK1 and LIMK2) phosphorylate cofilin and act as regulators of actin-myosin cytoskeletal dynamics, which result in actin polymerization, filament assemble, and stress fiber formation in smooth muscle cells. This may suggest that LIMKs promote smooth muscle contraction, however, not any associated study has been conducted. In this project, we aimed to explore the effects of LIMK inhibitors on prostate smooth muscle contraction.

Human prostate tissues were obtained from patients who underwent radical prostatectomy. RT-PCR, western blot and immunofluorescence were performed to detect LIMK in smooth muscle cells of prostate tissues. Phosphorylation of cofilin, a LIMK substrate, was detected by a phospho-specific antibody. Effects of LIMK inhibitors on smooth muscle contraction of prostate strips were performed with organ bath.

Expression of LIMK in smooth muscle cells of prostate tissues was suggested by RT-PCR, Western blot and immunofluorescence, while higher expression

level of LIMK was detected in prostate tissues than that in WPMY-1 cells. Two LIMK inhibitors, SR7826 (1 μM) and LIMKi3 (1 μM), showed significant effects on inhibiting contractions of prostate strips, which were induced by the α_1-adrenoceptor agonists, noradrenaline, phenylephrine and methoxamine, by the thromboxane A_2 analogue, U46619, and by EFS. Reduced phosphorylation of cofilin in prostate tissues treated with inhibitors was observed, which confirmed LIMK inhibition by SR7826 and LIMKi3. In WPMY-1 cells, a line of cultured cells from the prostate stroma, SR7826 and LIMKi3 were observed to cause breakdown of actin filaments and reduced viability in a concentration-dependent manner.

Together, this is the first study to explore the effects of small molecule LIMK inhibitors on regulating prostate smooth muscle contraction. The present study suggested that LIMKs promote prostate smooth muscle contraction by phosphorylating cofilin and subsequent causing actin organization, which could be inhibited by small molecule LIMK inhibitors, SR7826 and LIMKi3. Therefore, this project provides a possible novel therapy target for LUTS, although in vivo studies using animal models would be still warranted before clinical application.

8. Zusammenfassung

Der Sammelbegriff LUTS beschreibt eine Gruppe urologischer Beschwerden beim Wasserlassen mit vielfältiger Ätiologie. Ihre Prävalenz steigt mit dem Alter der Patientinnen und Patienten, und stellt auf Grund der demographischen Bevölkerungsentwicklung einen erheblichen sozioökonomischen Faktor von stark zunehmender Bedeutung dar. Bei Männern mit einer gutartigen Prostatavergrößerung (Benigne Prostatahyperplasie, BPH) führt ein erhöhter glattmuskulärer Tonus in der hyperplastischen Prostata häufig zu einer Verengung der Harnröhre und verursacht so eine Blasenauslassstörung (bladder outlet obstruction, BOO), und hierdurch zu Beeinträchtigungen der Blasenentleerung und Beschwerden beim Wasserlassen. Folglich zielen die Optionen der ersten Wahl zur medikamentösen Behandlung auf eine Hemmung der glattmuskulären Kontraktion in der Prostata ab. Die Effektivität der zur Verfügung stehenden Medikamente ist jedoch stark begrenzt, woraus sich ein dringender Bedarf an einem verbessertes Verständnis der Kontraktionsmechanismen und an der Identifizierung möglicher neuer Angriffspunkte ergibt. Verschiedene Studien zeigten, dass die LIM Kinasen (LIMK) über die Phosphorylierung von Cofilin in glatten Muskelzellen die Polymerisation von Aktin und dessen Filamentbildung fördern, was eine unabdingbare Voraussetzung der glattmuskulären Kontraktion darstellt. Dies legt zwar eine Rolle der LIMK für die glattmuskuläre Kontraktion nahe, was erstaunlicherweise bislang jedoch nie gezeigt wurde. In der vorliegenden Arbeit wurden daher die Effekte von LIMK-Inhibitoren auf die glattmuskuläre Kontraktion humaner Prostatagewebe untersucht.

Die hier verwendeten Prostatagewebe wurden im Rahmen von Tumor-bedingten, radikalen Prostatektomien gewonnen. Zur Detektion einer

möglichen LIMK-Expression wurden RT-PCRs, Western-Blot Analysen und Fluoreszenz-Färbungen durchgeführt. Phosphoryliertes Cofilin wurde mit einem phospho-spezifischen Antikörper detektiert. Die Effekte von LIMK-Inhibitoren auf die Kontraktion von Prostatageweben wurden in myographischen Messungen in einem Organbad untersucht. Ergänzend wurden Untersuchungen in WPMY-1-Zellen durchgeführt, einer Zelllinie aus dem Stroma einer humanen Prostata.

Die Ergebnisse aus RT-PCR, Western-Blot Analysen und Fluoreszenz-Färbungen legten eine LIMK-Expression in humanen Prostata-Geweben nahe, wobei LIMK1 offenbar in den glatten Muskelzellen des Stromas vorkommt. Diese Gewebe zeigten höhere Expressions-Level als WPMY-1-Zellen. Zwei strukturell unterschiedliche LIMK-Inhibitoren, SR7826 (1 µM) und LIMKi3 (1 µM) führten zu signifikanten Hemmung der Kontraktion von Prostata-Geweben, welche durch die α1-Adrenozeptor-Agonisten Noradrenalin, Phenylephrin und Methoxamin, sowie durch das Thromboxan-Analogon U46619, bzw. durch Ausschüttung endogener Neurotransmitter nach elektrischer Feldstimulation (EFS) ausgelöst wurden. Die Hemmung der LIMK durch SR7826 und LIMKi3 in Prostatageweben wurde durch eine Verminderung der Cofilin-Phosphorylierung bestätigt. In WPMY-1-Zellen verursachten SR7826 und LIMKi3 Konzentrations-abhängig einen Zusammenbruch der Aktin-Filamente und der Aktin-Polymerisation, sowie eine Verminderung der Viabilität.

Dies stellt die vermutlich erste Studie dar, welche eine Hemmung der glattmuskulären (Prostata-)Kontraktion durch LIMK-Inhibitoren zeigt. Die Ergebnisse legen nahe, dass LIMK die glattmuskuläre Kontraktion in der Prostata antreiben, was durch eine Phosphorylierung von Cofilin und eine daraus resultierende Bildung oder Aufrechterhaltung von Aktin-Filamenten

erfolgt. Sowohl die glattmuskuläre Kontraktion als auch die Organisation der Aktin-Filamente ließen sich in der Prostata durch SR7826 und LIMKi3 hemmen. Daher könnten LIM Kinasen einen neuen Angriffspunkt für mögliche neue LUTS-Therapien darstellen; jedoch sind in vivo Studien in Tiermodellen erforderlich, bevor diese Hemmstoffe in klinischen Studien verabreicht werden können.

9 References

Abrams P (1994) New words for old: lower urinary tract symptoms for "prostatism". Bmj 308:929-930

Abrams P, Cardozo L, Fall M, Griffiths D, Rosier P, Ulmsten U, van Kerrebroeck P, Victor A, Wein A (2002) The standardisation of terminology of lower urinary tract function: report from the Standardisation Sub-committee of the International Continence Society. Neurourology and urodynamics 21:167-178

Abrams P, Kaplan S, De Koning Gans HJ, Millard R (2006) Safety and tolerability of tolterodine for the treatment of overactive bladder in men with bladder outlet obstruction. The Journal of urology 175:999-1004; discussion 1004

Acevedo K, Moussi N, Li R, Soo P, Bernard O (2006) LIM kinase 2 is widely expressed in all tissues. The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society 54:487-501

Afeli SA, Rovner ES, Petkov GV (2013) BRL37344, a beta3-adrenergic receptor agonist, decreases nerve-evoked contractions in human detrusor smooth muscle isolated strips: role of BK channels. Urology 82:744.e741-747

Al-Ghabkari A, Deng JT, McDonald PC, Dedhar S, Alshehri M, Walsh MP, MacDonald JA (2016) A novel inhibitory effect of oxazol-5-one compounds on ROCKII signaling in human coronary artery vascular smooth muscle cells. Scientific reports 6:32118

Alcaraz A, Hammerer P, Tubaro A, Schroder FH, Castro R (2009) Is there evidence of a relationship between benign prostatic hyperplasia and prostate cancer? Findings of a literature review. European urology 55:864-873

Alexandre EC, Kiguti LR, Calmasini FB, Silva FH, da Silva KP, Ferreira R, Ribeiro CA, Monica FZ, Pupo AS, Antunes E (2016) Mirabegron relaxes urethral smooth muscle by a dual mechanism involving beta3 -adrenoceptor activation and alpha1 -adrenoceptor blockade. British journal of pharmacology 173:415-428

Andersson KE, Lepor H, Wyllie MG (1997) Prostatic alpha 1-adrenoceptors and uroselectivity. The Prostate 30:202-215

Andriole G, Bruchovsky N, Chung LW, Matsumoto AM, Rittmaster R, Roehrborn C, Russell D, Tindall D (2004) Dihydrotestosterone and the prostate: the scientific rationale for 5alpha-reductase inhibitors in the treatment of benign prostatic hyperplasia. The Journal of urology 172:1399-1403

Apostolidis A, Averbeck MA, Sahai A, Rahnama'i MS, Anding R, Robinson D, Gravas S, Dmochowski R (2017) Can we create a valid treatment algorithm for patients with drug resistant overactive bladder (OAB) syndrome or detrusor overactivity (DO)? Results from a think tank (ICI-RS 2015). Neurourology and urodynamics 36:882-893

Arruzazabala ML, Mas R, Molina V, Noa M, Carbajal D, Mendoza N (2006) Effect of D-004, a lipid extract from the Cuban royal palm fruit, on atypical prostate hyperplasia induced by phenylephrine in rats. Drugs in R&D 7:233-241

Bagheri-Yarmand R, Mazumdar A, Sahin AA, Kumar R (2006) LIM kinase 1 increases tumor metastasis of human breast cancer cells via regulation of the urokinase-type plasminogen activator system. International journal of cancer 118:2703-2710

Bansal A, Yadav P (2016) Foreign Bodies in the Urinary Bladder and Their Management: A Single-Centre Experience From North India. 20:260-269

Bergdahl S, Aus G, Lodding P, Norlen L, Hugosson J (1998) Lower urinary tract symptoms. A comparison of micturition symptoms in patients scheduled for transurethral prostatic resection and outpatients of both sexes referred for non-urological complaints. Scandinavian journal of urology and nephrology 32:20-25

Bernard O (2007) Lim kinases, regulators of actin dynamics. The international journal of biochemistry & cell biology 39:1071-1076

Berry SJ, Coffey DS, Walsh PC, Ewing LL (1984) The development of human benign prostatic hyperplasia with age. The Journal of urology 132:474-479

Biers SM, Reynard JM, Brading AF (2006) The effects of a new selective beta3-adrenoceptor agonist (GW427353) on spontaneous activity and detrusor relaxation in human bladder. BJU international 98:1310-1314

Bongalon S, Dai YP, Singer CA, Yamboliev IA (2004) PDGF and IL-1beta upregulate cofilin and LIMK2 in canine cultured pulmonary artery smooth muscle cells. Journal of vascular research 41:412-421

Bouallegue A, Daou GB, Srivastava AK (2007) Endothelin-1-induced signaling pathways in vascular smooth muscle cells. Current vascular pharmacology 5:45-52

Boyle P, Gould AL, Roehrborn CG (1996) Prostate volume predicts outcome of treatment of benign prostatic hyperplasia with finasteride: meta-analysis of randomized clinical trials. Urology 48:398-405

Boyle P, Robertson C, Mazzetta C, Keech M, Hobbs FD, Fourcade R, Kiemeney L, Lee C (2003a) The prevalence of male urinary incontinence in four centres: the UREPIK study. BJU international 92:943-947

Boyle P, Robertson C, Mazzetta C, Keech M, Hobbs FD, Fourcade R, Kiemeney L, Lee C (2003b) The prevalence of lower urinary tract symptoms in men and women in four centres. The UrEpik study. BJU international 92:409-414

Brandli A, Simpson JS, Ventura S (2010) Isoflavones isolated from red clover (Trifolium pratense) inhibit smooth muscle contraction of the isolated rat prostate gland. Phytomedicine : international journal of phytotherapy and phytopharmacology 17:895-901

Buljubasich R, Ventura S (2004) Adenosine 5'-triphosphate and noradrenaline are excitatory cotransmitters to the fibromuscular stroma of the guinea pig prostate gland. European journal of pharmacology 499:335-344

Buono R, Briganti A, Freschi M, Villa L, La Croce G, Moschini M, Benigni F, Castiglione F, Montorsi F, Hedlund P (2014) Silodosin and tadalafil have synergistic inhibitory effects on nerve-mediated contractions of human and rat isolated prostates. European journal of pharmacology 744:42-51

Cai S, Chen R, Li X, Cai Y, Ye Z, Li S, Li J, Huang H, Peng S, Wang J, Tao Y, Huang H, Wen X, Mo J, Deng Z, Wang J, Zhang Y, Gao X, Wen X (2015) Downregulation of microRNA-23a suppresses prostate cancer metastasis by targeting the PAK6-LIMK1 signaling pathway. Oncotarget 6:3904-3917

Capasso R, Aviello G, Borrelli F, Romano B, Ferro M, Castaldo L, Montanaro V, Altieri V, Izzo AA (2011) Inhibitory effect of standardized cannabis sativa extract and its ingredient cannabidiol on rat and human bladder contractility. Urology 77:1006.e1009-1006.e1015

Capasso R, Borrelli F, Capasso F, Mascolo N, Izzo AA (2004) Inhibitory effect of the antidepressant St.

John's wort (hypericum perforatum) on rat bladder contractility in vitro. Urology 64:168-172

Chapple C (2011) Overview on the lower urinary tract. Handbook of experimental pharmacology:1-14

Chapple CR, Abrams P, Andersson KE, Radziszewski P, Masuda T, Small M, Kuwayama T, Deacon S (2014) Phase II study on the efficacy and safety of the EP1 receptor antagonist ONO-8539 for nonneurogenic overactive bladder syndrome. The Journal of urology 191:253-260

Chapple CR, Montorsi F, Tammela TL, Wirth M, Koldewijn E, Fernandez Fernandez E (2011) Silodosin therapy for lower urinary tract symptoms in men with suspected benign prostatic hyperplasia: results of an international, randomized, double-blind, placebo- and active-controlled clinical trial performed in Europe. European urology 59:342-352

Chapple CR, Wein AJ, Abrams P, Dmochowski RR, Giuliano F, Kaplan SA, McVary KT, Roehrborn CG (2008) Lower urinary tract symptoms revisited: a broader clinical perspective. European urology 54:563-569

Chuang YC, Tyagi P, Huang CC, Chancellor MB, Yoshimura N (2012) Mechanisms and urodynamic effects of a potent and selective EP4 receptor antagonist, MF191, on cyclophosphamide and prostaglandin E2-induced bladder overactivity in rats. BJU international 110:1558-1564

Cindolo L, Pirozzi L, Fanizza C, Romero M, Tubaro A, Autorino R, De Nunzio C, Schips L (2015) Drug adherence and clinical outcomes for patients under pharmacological therapy for lower urinary tract symptoms related to benign prostatic hyperplasia: population-based cohort study. European urology 68:418-425

Cioanta I, Muschter R (2000) Water-induced thermotherapy for benign prostatic hyperplasia. Techniques in urology 6:294-299

Coyne KS, Kaplan SA, Chapple CR, Sexton CC, Kopp ZS, Bush EN, Aiyer LP (2009) Risk factors and comorbid conditions associated with lower urinary tract symptoms: EpiLUTS. BJU international 103 Suppl 3:24-32

Dai YP, Bongalon S, Mutafova-Yambolieva VN, Yamboliev IA (2008) Distinct effects of contraction agonists on the phosphorylation state of cofilin in pulmonary artery smooth muscle. Advances in pharmacological sciences 2008:362741

Dambros M, van Deutekom M, de Jongh R, van Koeveringe GA, De Mey JG, van Kerrebroeck P (2005) The inhibitory effect of the flavonoid galangin on urinary bladder smooth muscle contractility is mediated in part by modulation of Ca2+ release from intracellular stores. Planta medica 71:962-964

Davila M, Frost AR, Grizzle WE, Chakrabarti R (2003) LIM kinase 1 is essential for the invasive growth of prostate epithelial cells: implications in prostate cancer. The Journal of biological chemistry 278:36868-36875

Djavan B, Chapple C, Milani S, Marberger M (2004a) State of the art on the efficacy and tolerability of alpha1-adrenoceptor antagonists in patients with lower urinary tract symptoms suggestive of benign prostatic hyperplasia. Urology 64:1081-1088

Djavan B, Fong YK, Harik M, Milani S, Reissigl A, Chaudry A, Anagnostou T, Bagheri F, Waldert M, Kreuzer S, Fajkovic H, Marberger M (2004b) Longitudinal study of men with mild symptoms of bladder outlet obstruction treated with watchful waiting for four years. Urology 64:1144-1148

Doggweiler R, Whitmore KE, Meijlink JM, Drake MJ, Frawley H, Nordling J, Hanno P, Fraser MO, Homma Y, Garrido G, Gomes MJ, Elneil S, van de Merwe JP, Lin ATL, Tomoe H (2017) A standard for terminology in chronic pelvic pain syndromes: A report from the chronic pelvic

pain working group of the international continence society. Neurourology and urodynamics 36:984-1008

Dougherty PJ, Nepiyushchikh ZV, Chakraborty S, Wang W, Davis MJ, Zawieja DC, Muthuchamy M (2014) PKC activation increases Ca(2)(+) sensitivity of permeabilized lymphatic muscle via myosin light chain 20 phosphorylation-dependent and -independent mechanisms. American journal of physiology Heart and circulatory physiology 306:H674-683

Drake MJ (2014) Do we need a new definition of the overactive bladder syndrome? ICI-RS 2013. Neurourology and urodynamics 33:622-624

Du H, Wang X, Wu J, Qian Q (2010) Phenylephrine induces elevated RhoA activation and smooth muscle alpha-actin expression in Pkd2+/- vascular smooth muscle cells. Hypertension research : official journal of the Japanese Society of Hypertension 33:37-42

Elliott RA, Tonnu A, Ghaffar N, Taylor AH, Tincello DG, Norman RI (2013) Enhanced purinergic contractile responses and P2X1 receptor expression in detrusor muscle during cycles of hypoxia-glucopenia and reoxygenation. Experimental physiology 98:1683-1695

England RC, Norman RI, Elliott RA (2004) Direct inhibition of rat detrusor muscle contraction by erythromycin. Neurourology and urodynamics 23:273-279

Foletta VC, Moussi N, Sarmiere PD, Bamburg JR, Bernard O (2004) LIM kinase 1, a key regulator of actin dynamics, is widely expressed in embryonic and adult tissues. Experimental cell research 294:392-405

Fouda AM, Daba MH, Dahab GM (2007) Inhibitory effects of aqueous extract of Hibiscus sabdariffa on contractility of the rat bladder and uterus. Canadian journal of physiology and pharmacology 85:1020-1031

Fullhase C, Chapple C, Cornu JN, De Nunzio C, Gratzke C, Kaplan SA, Marberger M, Montorsi F, Novara G, Oelke M, Porst H, Roehrborn C, Stief C, McVary KT (2013) Systematic review of combination drug therapy for non-neurogenic male lower urinary tract symptoms. European urology 64:228-243

Furer K, Eberli D, Betschart C, Brenneisen R, De Mieri M, Hamburger M, Mennet-von Eiff M, Potterat O, Schnelle M, Simoes-Wust AP, von Mandach U (2015) Inhibition of porcine detrusor contractility by the flavonoid fraction of Bryophyllum pinnatum--a potential phytotherapeutic drug for the treatment of the overactive bladder syndrome. Phytomedicine : international journal of phytotherapy and phytopharmacology 22:158-164

Gandaglia G, Strittmatter F, La Croce G, Benigni F, Bettiga A, Castiglione F, Moschini M, Mistretta F, Gratzke C, Montorsi F, Stief C, Hedlund P (2014) The fatty acid amide hydrolase inhibitor oleoyl ethyl amide counteracts bladder overactivity in female rats. Neurourology and urodynamics 33:1251-1258

Garraway WM, Collins GN, Lee RJ (1991) High prevalence of benign prostatic hypertrophy in the community. Lancet (London, England) 338:469-471

Gerthoffer WT, Gunst SJ (2001) Invited review: focal adhesion and small heat shock proteins in the regulation of actin remodeling and contractility in smooth muscle. Journal of applied physiology (Bethesda, Md : 1985) 91:963-972

Gratzke C, Bachmann A, Descazeaud A, Drake MJ, Madersbacher S, Mamoulakis C, Oelke M, Tikkinen KA, Gravas S (2015) EAU Guidelines on the Assessment of Non-neurogenic Male Lower Urinary Tract Symptoms including Benign Prostatic Obstruction. European urology 67:1099-1109

Gratzke C, Weinhold P, Reich O, Seitz M, Schlenker B, Stief CG, Andersson KE, Hedlund P (2010) Transient receptor potential A1 and cannabinoid receptor activity in human normal and hyperplastic prostate: relation to nerves and interstitial cells. European urology 57:902-910

Guirgis FW, Khadpe JD, Kuntz GM, Wears RL, Kalynych CJ, Jones AE (2014) Persistent organ dysfunction after severe sepsis: a systematic review. Journal of critical care 29:320-326

Guneyli S, Ward E, Peng Y, Nehal Yousuf A, Trilisky I, Westin C, Antic T, Oto A (2017) MRI evaluation of benign prostatic hyperplasia: Correlation with international prostate symptom score. Journal of magnetic resonance imaging : JMRI 45:917-925

Gunst SJ, Zhang W (2008) Actin cytoskeletal dynamics in smooth muscle: a new paradigm for the regulation of smooth muscle contraction. American journal of physiology Cell physiology 295:C576-587

Hammarsten J, Peeker R (2011) Urological aspects of the metabolic syndrome. Nature reviews Urology 8:483-494

He Q, Wang Z, Liu G, Daneshgari F, MacLennan GT, Gupta S (2016) Metabolic syndrome, inflammation and lower urinary tract symptoms: possible translational links. Prostate cancer and prostatic diseases 19:7-13

Hedges JC, Dechert MA, Yamboliev IA, Martin JL, Hickey E, Weber LA, Gerthoffer WT (1999) A role for p38(MAPK)/HSP27 pathway in smooth muscle cell migration. The Journal of biological chemistry 274:24211-24219

Hennenberg M, Acevedo A, Wiemer N, Kan A, Tamalunas A, Wang Y, Yu Q, Rutz B, Ciotkowska A, Herlemann A, Strittmatter F, Stief CG, Gratzke C (2017a) Non-Adrenergic, Tamsulosin-Insensitive Smooth Muscle Contraction is Sufficient to Replace alpha1 -Adrenergic Tension in the Human Prostate. The Prostate 77:697-707

Hennenberg M, Biecker E, Trebicka J, Jochem K, Zhou Q, Schmidt M, Jakobs KH, Sauerbruch T, Heller J (2006) Defective RhoA/Rho-kinase signaling contributes to vascular hypocontractility and vasodilation in cirrhotic rats. Gastroenterology 130:838-854

Hennenberg M, Miljak M, Herrmann D, Strittmatter F, Walther S, Rutz B, Hocaoglu Y, Kunit T, Schreiber A, Andersson KE, Stief CG, Gratzke C (2013) The receptor antagonist picotamide inhibits adrenergic and thromboxane-induced contraction of hyperplastic human prostate smooth muscle. American journal of physiology Renal physiology 305:F1383-1390

Hennenberg M, Schott M, Kan A, Keller P, Tamalunas A, Ciotkowska A, Rutz B, Wang Y, Strittmatter F, Herlemann A, Yu Q, Stief CG, Gratzke C (2016) Inhibition of Adrenergic and Non-Adrenergic Smooth Muscle Contraction in the Human Prostate by the Phosphodiesterase 10-Selective Inhibitor TC-E 5005. The Prostate 76:1364-1374

Hennenberg M, Stief CG, Gratzke C (2014a) Pharmacology of the lower urinary tract. Indian journal of urology : IJU : journal of the Urological Society of India 30:181-188

Hennenberg M, Stief CG, Gratzke C (2014b) Prostatic alpha1-adrenoceptors: new concepts of function, regulation, and intracellular signaling. Neurourology and urodynamics 33:1074-1085

Hennenberg M, Tamalunas A, Wang Y, Keller P, Schott M, Strittmatter F, Herlemann A, Yu Q, Rutz B, Ciotkowska A, Stief CG, Gratzke C (2017b) Inhibition of agonist-induced smooth muscle contraction by picotamide in the male human lower urinary tract outflow region. European journal of pharmacology 803:39-47

Hennenberg M, Trebicka J, Sauerbruch T, Heller J (2008) Mechanisms of extrahepatic vasodilation in portal hypertension. Gut 57:1300-1314

Herlemann A, Keller P, Schott M, Tamalunas A, Ciotkowska A, Rutz B, Wang Y, Yu Q, Waidelich R, Strittmatter F, Stief CG, Gratzke C, Hennenberg M (2017) Inhibition of smooth muscle contraction and ARF 6 activity by the inhibitor for cytohesin GEFs, secinH3 in the human prostate. American journal of physiology Renal physiology:ajprenal.00125.02017

Herschorn S, Jones JS, Oelke M, MacDiarmid S, Wang JT, Guan Z (2010) Efficacy and tolerability of fesoterodine in men with overactive bladder: a pooled analysis of 2 phase III studies. Urology 75:1149-1155

Hollingsworth JM, Wilt TJ (2014) Lower urinary tract symptoms in men. Bmj 349:g4474

Hong SK, Kwak C, Chang Jeong B, Kim BS, Kim HH (2005) Involvement of Rho-kinase in the contractile mechanism of human ureteral smooth muscle. Neurourology and urodynamics 24:136-141

Hypolite JA, Chang S, Wein AJ, Chacko S, Malykhina AP (2015) Protein kinase C modulates frequency of micturition and non-voiding contractions in the urinary bladder via neuronal and myogenic mechanisms. BMC urology 15:34

Irwin DE, Kopp ZS, Agatep B, Milsom I, Abrams P (2011) Worldwide prevalence estimates of lower urinary tract symptoms, overactive bladder, urinary incontinence and bladder outlet obstruction. BJU international 108:1132-1138

Irwin DE, Milsom I, Hunskaar S, Reilly K, Kopp Z, Herschorn S, Coyne K, Kelleher C, Hampel C, Artibani W, Abrams P (2006) Population-based survey of urinary incontinence, overactive bladder, and other lower urinary tract symptoms in five countries: results of the EPIC study. European urology 50:1306-1314; discussion 1314-1305

Irwin DE, Milsom I, Kopp Z, Abrams P, Artibani W, Herschorn S (2009) Prevalence, severity, and symptom bother of lower urinary tract symptoms among men in the EPIC study: impact of overactive bladder. European urology 56:14-20

Ishigooka M, Hashimoto T, Suzuki Y, Ichiyanagi O, Sasagawa I, Aoyama N, Nakada T (1997) Functional property, norepinephrine content and morphometric findings in human hyperplastic prostate. The Prostate 33:183-187

Ito Y, Seki M, Nishioka Y, Kimura M, Yasuda A, Kirimoto T, Yasuda T, Yamada S (2009) Pharmacological effects of Hachi-mi-jio-gan extract (Harncare) on the contractile response and on pharmacologically relevant receptors in the rat bladder. Yakugaku zasshi : Journal of the Pharmaceutical Society of Japan 129:957-964

Izett M, Zacche M, Thiagamoorthy G, Robinson D, Cardozo L (2017) Current evidence and emerging drug therapies for overactive bladder. Minerva ginecologica 69:269-285

Kang SG, Park CH, Kim DK, Park JK, Hong SJ, Chung BH, Kim CS, Lee KS, Kim JC, Lee JG (2011) Long-term outcome of tamsulosin for patients with lower urinary tract symptoms according to the treatment response defined by lower urinary tract symptom outcomes score. International journal of clinical practice 65:691-697

Kaplan SA, Goldfischer ER, Steers WD, Gittelman M, Andoh M, Forero-Schwanhaeuser S (2010) Solifenacin treatment in men with overactive bladder: effects on symptoms and patient-reported outcomes. The aging male : the official journal of the International Society for the Study of the Aging Male 13:100-107

Kedia GT, Uckert S, Kedia M, Kuczyk MA (2009) Effects of phosphodiesterase inhibitors on contraction induced by endothelin-1 of isolated human prostatic tissue. Urology 73:1397-1401

Kedia GT, Uckert S, Polat H, Merseburger AS, Kuczyk MA (2012) Evaluating the significance of cyclic adenosine monophosphate-mediated signaling in human prostate: a functional and

biochemical study. Urology 80:952.e959-914

Kim HJ, Yang HM, Kim DH, Kim HG, Jang WC, Lee YR (2003) Effects of ylang-ylang essential oil on the relaxation of rat bladder muscle in vitro and white rabbit bladder in vivo. Journal of Korean medical science 18:409-414

Kim TN, Nam JK, Lee KS, Kim TH, Park SW, Shin DG, Park HJ, Lee W, Lee ZZ, Chung MK (2014) Reasons for prescription change of alpha1-blockers in patients with lower urinary tract symptoms suggestive of benign prostatic hyperplasia. Urology 84:427-432

Kirby RS, Roehrborn C, Boyle P, Bartsch G, Jardin A, Cary MM, Sweeney M, Grossman EB (2003) Efficacy and tolerability of doxazosin and finasteride, alone or in combination, in treatment of symptomatic benign prostatic hyperplasia: the Prospective European Doxazosin and Combination Therapy (PREDICT) trial. Urology 61:119-126

Kirschstein T, Protzel C, Porath K, Sellmann T, Kohling R, Hakenberg OW (2014) Age-dependent contribution of Rho kinase in carbachol-induced contraction of human detrusor smooth muscle in vitro. Acta pharmacologica Sinica 35:74-81

Kirschstein T, Sahre T, Kernig K, Protzel C, Porath K, Kohling R, Hakenberg OW (2015) Inverse relationship of Rho kinase and myosin-light chain kinase expression in the aging human detrusor smooth muscle. BMC urology 15:104

Kitazawa T, Kobayashi S, Horiuti K, Somlyo AV, Somlyo AP (1989) Receptor-coupled, permeabilized smooth muscle. Role of the phosphatidylinositol cascade, G-proteins, and modulation of the contractile response to Ca2+. The Journal of biological chemistry 264:5339-5342

Kondo S, Tashima Y, Morita T (1993) Quantitative analysis of adrenergic alpha-1 and alpha-2 receptors in human prostatic urethral tissue. British journal of urology 72:68-73

Koskimaki J, Hakama M, Huhtala H, Tammela TL (2001) Association of non-urological diseases with lower urinary tract symptoms. Scandinavian journal of urology and nephrology 35:377-381

Kowalczyk J (2003) A review of the overactive bladder in women and men. The journal of gender-specific medicine : JGSM : the official journal of the Partnership for Women's Health at Columbia 6:25-29

Kramer G, Mitteregger D, Marberger M (2007) Is benign prostatic hyperplasia (BPH) an immune inflammatory disease? European urology 51:1202-1216

Kunit T, Gratzke C, Schreiber A, Strittmatter F, Waidelich R, Rutz B, Loidl W, Andersson KE, Stief CG, Hennenberg M (2014) Inhibition of smooth muscle force generation by focal adhesion kinase inhibitors in the hyperplastic human prostate. American journal of physiology Renal physiology 307:F823-832

Lee CL, Kuo HC (2017a) Current consensus and controversy on the diagnosis of male lower urinary tract symptoms/benign prostatic hyperplasia. Ci ji yi xue za zhi = Tzu-chi medical journal 29:6-11

Lee CL, Kuo HC (2017b) Pathophysiology of benign prostate enlargement and lower urinary tract symptoms: Current concepts. Ci ji yi xue za zhi = Tzu-chi medical journal 29:79-83

Lee HN, Lee KS, Kim JC, Chung BH, Kim CS, Lee JG, Kim DK, Park CH, Park JK, Hong SJ (2015) Rate and associated factors of solifenacin add-on after tamsulosin monotherapy in men with voiding and storage lower urinary tract symptoms. International journal of clinical practice 69:444-453

Lee YS, Lee KS, Jung JH, Han DH, Oh SJ, Seo JT, Lee JG, Park HS, Choo MS (2011) Prevalence of overactive bladder, urinary incontinence, and lower urinary tract symptoms: results of Korean

EPIC study. World journal of urology 29:185-190

Lepor H (2005) Pathophysiology of benign prostatic hyperplasia in the aging male population. Reviews in urology 7 Suppl 4:S3-s12

Levin RM, Hass MA, Bellamy F, Horan P, Whitbeck K, Chow PH, Kung LS, Gosling J (2002) Effect of oral Tadenan treatment on rabbit bladder structure and function after partial outlet obstruction. The Journal of urology 167:2253-2259

Levitt JM, Slawin KM (2007) Prostate-specific antigen and prostate-specific antigen derivatives as predictors of benign prostatic hyperplasia progression. Current urology reports 8:269-274

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods (San Diego, Calif) 25:402-408

MacKenzie KR, Aning JJ (2016) Managing lower urinary tract symptoms in men. The Practitioner 260:11-16, 12

Manjunatha R, Pundarikaksha HP, Madhusudhana HR, Amarkumar J, Hanumantharaju BK (2016) A randomized, comparative, open-label study of efficacy and tolerability of alfuzosin, tamsulosin and silodosin in benign prostatic hyperplasia. Indian journal of pharmacology 48:134-140

Mardilovich K, Gabrielsen M, McGarry L, Orange C, Patel R, Shanks E, Edwards J, Olson MF (2015) Elevated LIM kinase 1 in nonmetastatic prostate cancer reflects its role in facilitating androgen receptor nuclear translocation. Molecular cancer therapeutics 14:246-258

Mastrangelo D, Wisard M, Rohner S, Leisinger H, Iselin CE (2000) Diclofenac and NS-398, a selective cyclooxygenase-2 inhibitor, decrease agonist-induced contractions of the pig isolated ureter. Urological research 28:376-382

Matsukawa Y, Gotoh M, Komatsu T, Funahashi Y, Sassa N, Hattori R (2013) Efficacy of silodosin for relieving benign prostatic obstruction: prospective pressure flow study. The Journal of urology 189:S117-121

Matthiesen TB, Rittig S, Norgaard JP, Pedersen EB, Djurhuus JC (1996) Nocturnal polyuria and natriuresis in male patients with nocturia and lower urinary tract symptoms. The Journal of urology 156:1292-1299

McConnell JD, Bruskewitz R, Walsh P, Andriole G, Lieber M, Holtgrewe HL, Albertsen P, Roehrborn CG, Nickel JC, Wang DZ, Taylor AM, Waldstreicher J (1998) The effect of finasteride on the risk of acute urinary retention and the need for surgical treatment among men with benign prostatic hyperplasia. Finasteride Long-Term Efficacy and Safety Study Group. The New England journal of medicine 338:557-563

McDonald C, Winge K, Burn DJ (2017) Lower urinary tract symptoms in Parkinson's disease: Prevalence, aetiology and management. Parkinsonism & related disorders 35:8-16

Michel MC, Gravas S (2016) Safety and tolerability of beta3-adrenoceptor agonists in the treatment of overactive bladder syndrome - insight from transcriptosome and experimental studies. Expert opinion on drug safety 15:647-657

Michel MC, Mehlburger L, Bressel HU, Goepel M (1998) Comparison of tamsulosin efficacy in subgroups of patients with lower urinary tract symptoms. Prostate cancer and prostatic diseases 1:332-335

Milani S, Djavan B (2005) Lower urinary tract symptoms suggestive of benign prostatic hyperplasia: latest update on alpha-adrenoceptor antagonists. BJU international 95 Suppl 4:29-36

Min J, Reznichenko M, Poythress RH, Gallant CM, Vetterkind S, Li Y, Morgan KG (2012) Src modulates

contractile vascular smooth muscle function via regulation of focal adhesions. Journal of cellular physiology 227:3585-3592

Mistretta FA, Russo A, Castiglione F, Bettiga A, Colciago G, Montorsi F, Brandolini L, Aramini A, Bianchini G, Allegretti M, Bovolenta S, Russo R, Benigni F, Hedlund P (2016) DFL23448, A Novel Transient Receptor Potential Melastin 8-Selective Ion Channel Antagonist, Modifies Bladder Function and Reduces Bladder Overactivity in Awake Rats. The Journal of pharmacology and experimental therapeutics 356:200-211

Mokry J, Nosalova G, Jakubesova M (2005) Propantheline and in vitro reactivity of urinary bladder smooth muscle in guinea pigs. Bratislavske lekarske listy 106:151-154

Nakano K, Kanai-Azuma M, Kanai Y, Moriyama K, Yazaki K, Hayashi Y, Kitamura N (2003) Cofilin phosphorylation and actin polymerization by NRK/NESK, a member of the germinal center kinase family. Experimental cell research 287:219-227

Naslund MJ, Miner M (2007) A review of the clinical efficacy and safety of 5alpha-reductase inhibitors for the enlarged prostate. Clinical therapeutics 29:17-25

Nielsen KK, Nordling J, Hald T (1994) Critical review of the diagnosis of prostatic obstruction. Neurourology and urodynamics 13:201-217

Nitti VW, Rosenberg S, Mitcheson DH, He W, Fakhoury A, Martin NE (2013) Urodynamics and safety of the beta(3)-adrenoceptor agonist mirabegron in males with lower urinary tract symptoms and bladder outlet obstruction. The Journal of urology 190:1320-1327

Oelke M, Bachmann A, Descazeaud A, Emberton M, Gravas S, Michel MC, N'Dow J, Nordling J, de la Rosette JJ (2013) EAU guidelines on the treatment and follow-up of non-neurogenic male lower urinary tract symptoms including benign prostatic obstruction. European urology 64:118-140

Oelke M, Burger M, Castro-Diaz D, Chartier-Kastler E, Jimenez Cidre MA, McNicholas T, Radziszewski P, Kirby M (2012a) Diagnosis and medical treatment of lower urinary tract symptoms in adult men: applying specialist guidelines in clinical practice. BJU international 110:710-718

Oelke M, Giuliano F, Baygani SK, Melby T, Sontag A (2014) Treatment satisfaction with tadalafil or tamsulosin vs placebo in men with lower urinary tract symptoms (LUTS) suggestive of benign prostatic hyperplasia (BPH): results from a randomised, placebo-controlled study. BJU international 114:568-575

Oelke M, Giuliano F, Mirone V, Xu L, Cox D, Viktrup L (2012b) Monotherapy with tadalafil or tamsulosin similarly improved lower urinary tract symptoms suggestive of benign prostatic hyperplasia in an international, randomised, parallel, placebo-controlled clinical trial. European urology 61:917-925

Oelke M, Shinghal R, Sontag A, Baygani SK, Donatucci CF (2015) Time to onset of clinically meaningful improvement with tadalafil 5 mg once daily for lower urinary tract symptoms secondary to benign prostatic hyperplasia: analysis of data pooled from 4 pivotal, double-blind, placebo controlled studies. The Journal of urology 193:1581-1589

Oger S, Behr-Roussel D, Gorny D, Lebret T, Denoux Y, Alexandre L, Giuliano F (2010) Combination of alfuzosin and tadalafil exerts an additive relaxant effect on human detrusor and prostatic tissues in vitro. European urology 57:699-707

Onoruvwe O, Olayinka AO, Lot TY, Udoh FV (2001) Effects of stem bark and leaf extracts of Bridelia ferruginea on rat bladder smooth muscle. Fitoterapia 72:230-235

Orsted DD, Bojesen SE (2013) The link between benign prostatic hyperplasia and prostate cancer.

Nature reviews Urology 10:49-54

Pacher P, Batkai S, Kunos G (2006) The endocannabinoid system as an emerging target of pharmacotherapy. Pharmacological reviews 58:389-462

Park JB, Agnihotri S, Golbourn B, Bertrand KC, Luck A, Sabha N, Smith CA, Byron S, Zadeh G, Croul S, Berens M, Rutka JT (2014) Transcriptional profiling of GBM invasion genes identifies effective inhibitors of the LIM kinase-Cofilin pathway. Oncotarget 5:9382-9395

Peng TR, Lee MC, Wu TW, Lan CC (2014) Suspected ketamine-associated lower urinary tract symptoms. Urology journal 11:1508-1510

Petrilli A, Copik A, Posadas M, Chang LS, Welling DB, Giovannini M, Fernandez-Valle C (2014) LIM domain kinases as potential therapeutic targets for neurofibromatosis type 2. Oncogene 33:3571-3582

Porst H, Roehrborn CG, Secrest RJ, Esler A, Viktrup L (2013) Effects of tadalafil on lower urinary tract symptoms secondary to benign prostatic hyperplasia and on erectile dysfunction in sexually active men with both conditions: analyses of pooled data from four randomized, placebo-controlled tadalafil clinical studies. The journal of sexual medicine 10:2044-2052

Pradidarcheep W, Wallner C, Dabhoiwala NF, Lamers WH (2011) Anatomy and histology of the lower urinary tract. Handbook of experimental pharmacology:117-148

Price DT, Schwinn DA, Lomasney JW, Allen LF, Caron MG, Lefkowitz RJ (1993) Identification, quantification, and localization of mRNA for three distinct alpha 1 adrenergic receptor subtypes in human prostate. The Journal of urology 150:546-551

Rahnama'i MS, van Koeveringe GA, van Kerrebroeck PE, de Wachter SG (2013) The effect of indomethacin on the muscarinic induced contractions in the isolated normal guinea pig urinary bladder. BMC urology 13:8

Rittmaster RS, Norman RW, Thomas LN, Rowden G (1996) Evidence for atrophy and apoptosis in the prostates of men given finasteride. The Journal of clinical endocrinology and metabolism 81:814-819

Roehrborn CG (2006) Three months' treatment with the alpha1-blocker alfuzosin does not affect total or transition zone volume of the prostate. Prostate cancer and prostatic diseases 9:121-125

Roehrborn CG (2008) BPH progression: concept and key learning from MTOPS, ALTESS, COMBAT, and ALF-ONE. BJU international 101 Suppl 3:17-21

Roehrborn CG, Abrams P, Rovner ES, Kaplan SA, Herschorn S, Guan Z (2006) Efficacy and tolerability of tolterodine extended-release in men with overactive bladder and urgency urinary incontinence. BJU international 97:1003-1006

Roehrborn CG, Boyle P, Nickel JC, Hoefner K, Andriole G (2002) Efficacy and safety of a dual inhibitor of 5-alpha-reductase types 1 and 2 (dutasteride) in men with benign prostatic hyperplasia. Urology 60:434-441

Roehrborn CG, Kaplan SA, Kraus SR, Wang JT, Bavendam T, Guan Z (2008a) Effects of serum PSA on efficacy of tolterodine extended release with or without tamsulosin in men with LUTS, including OAB. Urology 72:1061-1067; discussion 1067

Roehrborn CG, Siami P, Barkin J, Damiao R, Major-Walker K, Morrill B, Montorsi F (2008b) The effects of dutasteride, tamsulosin and combination therapy on lower urinary tract symptoms in men with benign prostatic hyperplasia and prostatic enlargement: 2-year results from the CombAT study. The Journal of urology 179:616-621; discussion 621

Roehrborn CG, Siami P, Barkin J, Damiao R, Major-Walker K, Nandy I, Morrill BB, Gagnier RP, Montorsi

F (2010) The effects of combination therapy with dutasteride and tamsulosin on clinical outcomes in men with symptomatic benign prostatic hyperplasia: 4-year results from the CombAT study. European urology 57:123-131

Ross-Macdonald P, de Silva H, Guo Q, Xiao H, Hung CY, Penhallow B, Markwalder J, He L, Attar RM, Lin TA, Seitz S, Tilford C, Wardwell-Swanson J, Jackson D (2008) Identification of a nonkinase target mediating cytotoxicity of novel kinase inhibitors. Molecular cancer therapeutics 7:3490-3498

San Martin A, Lee MY, Williams HC, Mizuno K, Lassegue B, Griendling KK (2008) Dual regulation of cofilin activity by LIM kinase and Slingshot-1L phosphatase controls platelet-derived growth factor-induced migration of human aortic smooth muscle cells. Circulation research 102:432-438

Scott RW, Olson MF (2007) LIM kinases: function, regulation and association with human disease. Journal of molecular medicine (Berlin, Germany) 85:555-568

Seo YM, Kim HJ (2012) Impact of intravesical protrusion of the prostate in the treatment of lower urinary tract symptoms/benign prostatic hyperplasia of moderate size by alpha receptor antagonist. International neurourology journal 16:187-190

Shaikhibrahim Z, Lindstrot A, Ellinger J, Rogenhofer S, Buettner R, Perner S, Wernert N (2012) The peripheral zone of the prostate is more prone to tumor development than the transitional zone: is the ETS family the key? Molecular medicine reports 5:313-316

Somlyo AP, Somlyo AV (2004) Signal transduction through the RhoA/Rho-kinase pathway in smooth muscle. Journal of muscle research and cell motility 25:613-615

Stewart WF, Van Rooyen JB, Cundiff GW, Abrams P, Herzog AR, Corey R, Hunt TL, Wein AJ (2003) Prevalence and burden of overactive bladder in the United States. World journal of urology 20:327-336

Strittmatter F, Gratzke C, Weinhold P, Steib CJ, Hartmann AC, Schlenker B, Andersson KE, Hedlund P, Stief CG, Hennenberg M (2011) Thromboxane A2 induces contraction of human prostate smooth muscle by Rho kinase- and calmodulin-dependent mechanisms. European journal of pharmacology 650:650-655

Swinney DC (2004) Biochemical mechanisms of drug action: what does it take for success? Nature reviews Drug discovery 3:801-808

Takahashi R, Nishimura J, Seki N, Yunoki T, Tomoda T, Kanaide H, Naito S (2007) RhoA/Rho kinase-mediated Ca2+ sensitization in the contraction of human prostate. Neurourology and urodynamics 26:547-551

Takeda H, Matsuzawa A, Igawa Y, Yamazaki Y, Kaidoh K, Akahane S, Kojima M, Miyata H, Akahane M, Nishizawa O (2003) Functional characterization of beta-adrenoceptor subtypes in the canine and rat lower urinary tract. The Journal of urology 170:654-658

Taub DA, Wei JT (2006) The economics of benign prostatic hyperplasia and lower urinary tract symptoms in the United States. Current urology reports 7:272-281

Tokanovic S, Malone DT, Ventura S (2007) Stimulation of epithelial CB1 receptors inhibits contractions of the rat prostate gland. British journal of pharmacology 150:227-234

Tokanovic S, White CW, Malone DT, Exintaris B, Ventura S (2010) Characterisation of the prostanoid receptor mediating inhibition of smooth muscle contractility in the rat prostate gland. Naunyn-Schmiedeberg's archives of pharmacology 381:321-328

Toshima J, Toshima JY, Amano T, Yang N, Narumiya S, Mizuno K (2001a) Cofilin phosphorylation by

protein kinase testicular protein kinase 1 and its role in integrin-mediated actin reorganization and focal adhesion formation. Molecular biology of the cell 12:1131-1145

Toshima J, Toshima JY, Takeuchi K, Mori R, Mizuno K (2001b) Cofilin phosphorylation and actin reorganization activities of testicular protein kinase 2 and its predominant expression in testicular Sertoli cells. The Journal of biological chemistry 276:31449-31458

Turna B, Cinar MG, Canda AE, Orhan EC, Tiftik NR, Nazli O, Buyukafsar K (2007) Role of Rho-kinase in contractions of ureters from rabbits with unilateral ureteric obstruction. BJU international 100:1166-1171

Vahabi B, Wagg AS, Rosier P, Rademakers KLJ, Denys MA, Pontari M, Lovick T, Valentini FA, Nelson PP, Andersson KE, Fry CH (2017) Can we define and characterize the aging lower urinary tract?-ICI-RS 2015. Neurourology and urodynamics 36:854-858

Vaughan ED (2003) Long-Term Experience With 5-alpha-Reductase Inhibitors. Reviews in urology 5 Suppl 5:S22-27

Vauquelin G, Van Liefde I, Vanderheyden P (2002) Models and methods for studying insurmountable antagonism. Trends in pharmacological sciences 23:514-518

Ventura S, Dewalagama RK, Lau LC (2003) Adenosine 5'-triphosphate (ATP) is an excitatory cotransmitter with noradrenaline to the smooth muscle of the rat prostate gland. British journal of pharmacology 138:1277-1284

Walden PD, Gerardi C, Lepor H (1999) Localization and expression of the alpha1A-1, alpha1B and alpha1D-adrenoceptors in hyperplastic and non-hyperplastic human prostate. The Journal of urology 161:635-640

Walther S, Strittmatter F, Roosen A, Heinzer F, Rutz B, Stief CG, Gratzke C, Hennenberg M (2012) Expression and alpha1-adrenoceptor regulation of caldesmon in human prostate smooth muscle. Urology 79:745.e745-712

Wang M, Wei X, Shi L, Chen B, Zhao G, Yang H (2014) Integrative genomic analyses of the histamine H1 receptor and its role in cancer prediction. International journal of molecular medicine 33:1019-1026

Wang Y, Gratzke C, Tamalunas A, Rutz B, Ciotkowska A, Strittmatter F, Herlemann A, Janich S, Waidelich R, Liu C, Stief CG, Hennenberg M (2016a) Smooth muscle contraction and growth of stromal cells in the human prostate are both inhibited by the Src family kinase inhibitors, AZM475271 and PP2. British journal of pharmacology

Wang Y, Gratzke C, Tamalunas A, Wiemer N, Ciotkowska A, Rutz B, Waidelich R, Strittmatter F, Liu C, Stief CG, Hennenberg M (2016b) P21-Activated Kinase Inhibitors FRAX486 and IPA3: Inhibition of Prostate Stromal Cell Growth and Effects on Smooth Muscle Contraction in the Human Prostate. PloS one 11:e0153312

Wang Y, Kunit T, Ciotkowska A, Rutz B, Schreiber A, Strittmatter F, Waidelich R, Liu C, Stief CG, Gratzke C, Hennenberg M (2015) Inhibition of prostate smooth muscle contraction and prostate stromal cell growth by the inhibitors of Rac, NSC23766 and EHT1864. British journal of pharmacology 172:2905-2917

Webb RC (2003) Smooth muscle contraction and relaxation. Advances in physiology education 27:201-206

Weinhold P, Hennenberg M, Strittmatter F, Stief CG, Gratzke C, Hedlund P (2017) Transient receptor potential a1 (TRPA1) agonists inhibit contractions of the isolated human ureter. Neurourology and urodynamics

White CW, Short JL, Evans RJ, Ventura S (2015) Development of a P2X1-purinoceptor mediated contractile response in the aged mouse prostate gland through slowing down of ATP breakdown. Neurourology and urodynamics 34:292-298

White CW, Short JL, Ventura S (2013) Rho kinase activation mediates adrenergic and cholinergic smooth muscle contractile responses in the mouse prostate gland. European journal of pharmacology 721:313-321

Wilson JL, Rupasinghe C, Usheva A, Warburton R, Kaplan C, Taylor L, Hill N, Mierke DF, Polgar P (2015) Modulating the dysregulated migration of pulmonary arterial hypertensive smooth muscle cells with motif mimicking cell permeable peptides. Current topics in peptide & protein research 16:1-17

Xu Y, Ventura S (2010) Extracts of bark from the traditional Chinese herb Phellodendron amurense inhibit contractility of the isolated rat prostate gland. Journal of ethnopharmacology 127:196-199

Yamanishi T, Yasuda K, Kitahara S, Nakai H, Yoshida K, Iizuka H (2006) Effects of 138-355, a beta3-adrenoceptor selective agonist, on relaxation of the human detrusor muscle in vitro. Neurourology and urodynamics 25:815-819

Yang N, Higuchi O, Ohashi K, Nagata K, Wada A, Kangawa K, Nishida E, Mizuno K (1998) Cofilin phosphorylation by LIM-kinase 1 and its role in Rac-mediated actin reorganization. Nature 393:809-812

Yin Y, Zheng K, Eid N, Howard S, Jeong JH, Yi F, Guo J, Park CM, Bibian M, Wu W, Hernandez P, Park H, Wu Y, Luo JL, LoGrasso PV, Feng Y (2015) Bis-aryl urea derivatives as potent and selective LIM kinase (Limk) inhibitors. Journal of medicinal chemistry 58:1846-1861

Yokoyama T, Uematsu K, Watanabe T, Sasaki K, Kumon H, Nagai A (2009) Naftopidil and propiverine hydrochloride for treatment of male lower urinary tract symptoms suggestive of benign prostatic hyperplasia and concomitant overactive bladder: a prospective randomized controlled study. Scandinavian journal of urology and nephrology 43:307-314

Yuan Y, Tan YF, Xu P, Li H, Li YH, Chen WY, Zhang JQ, Chen F, Huang GJ (2014) Izalpinin from fruits of Alpinia oxyphylla with antagonistic activity against the rat bladder contractility. African journal of traditional, complementary, and alternative medicines : AJTCAM 11:120-125

Zhang X, Seftel A, DiSanto ME (2011) Blebbistain, a myosin II inhibitor, as a novel strategy to regulate detrusor contractility in a rat model of partial bladder outlet obstruction. PloS one 6:e25958

Zhao XH, Laschinger C, Arora P, Szaszi K, Kapus A, McCulloch CA (2007) Force activates smooth muscle alpha-actin promoter activity through the Rho signaling pathway. Journal of cell science 120:1801-1809

Zhou Q, Kee YS, Poirier CC, Jelinek C, Osborne J, Divi S, Surcel A, Will ME, Eggert US, Muller-Taubenberger A, Iglesias PA, Cotter RJ, Robinson DN (2010) 14-3-3 coordinates microtubules, Rac, and myosin II to control cell mechanics and cytokinesis. Current biology : CB 20:1881-1889

Zhu KC, Sun JM, Shen JG, Jin JZ, Liu F, Xu XL, Chen L, Liu LT, Lv JJ (2015) Afzelin exhibits anti-cancer activity against androgen-sensitive LNCaP and androgen-independent PC-3 prostate cancer cells through the inhibition of LIM domain kinase 1. Oncology letters 10:2359-2365

10. Abbreviations

5-ARIs	5a-reductase inhibitors
5-EdU	5-Ethynyl-deoxyuridine
5-HT	5-hydroxytryptamine
ADF	Actin depolymerizing factors
ANOVA	Analysis of variance
ATP	Adenosine 5'-triphosphate
Bim-1	Bisindolylmaleimide1
BOO	Bladder outlet obstruction
BPH	Benign prostatic hyperplasia
$CaCl_2 \cdot 2H_2O$	Calcium chloride dihydrate
cAMP	Cyclic adenosine monophosphate
CCK	Cell Counting Kit
Cdc42	Cell division cycle 42
COX	Cyclooxygenase
CP	Crossing points
Ct	Number of cycles
DAG	Diacylglycerol
DAPI	4',6'-diamidino-2-phenylindole-dihydrochloride
DAPI	4', 6'-diamidino-2-phenylindole-dihydrochloride
DG	Diacylglycerol
DHT	Dihydrotestosterone
DMSO	Dimethyl sulfoxide
DO	Detrusor overactivity
DTT	Dithiothreitol
DU	Detrusor underactivity
EC50	Half maximal effective concentration
ECL	Enhanced chemiluminescence
EDTA	Ethylenediaminetetraacetic acid
EFS	Electric field stimulation
EP1	Prostaglandin E_2 receptor subtype 1
EP_4	Prostaglandin E_2 receptor subtype 4
FAAH	Fatty acid amide hydrolase
FAK	Focal adhesion kinase
FCS	Fetal calf serum
FITC	Fluorescein isothiocyanate
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GPCR	G protein-coupled receptor

GTPase	Guanosine triphosphate kinase
HSP	Heat shock protein
IC50	Half maximal inhibitory concentration
IP3	Inositol 1,4,5-trisphosphate
IPSS	International Prostate Symptom Score
KCl	Potassium chloride
KH	Krebs- Henseleit solution
KH_2PO_4	Potassium hydrogen phosphate
LIMKs	LIM domain kinases
LUTS	Lower urinary tract symptoms
MAPK	Mitogen-activated protein kinase
$MgCl_2$	Magnesium chloride
$MgSO_4 \cdot 7H_2O$	Magnesium sulfate heptahydrate
MLC	Myosin light chain
MRCK	Cdc42-binding kinase
NaCl	Sodium chloride
$NaHCO_3$	Sodium bicarbonate
OAB	Overactive bladder
OCT	Optimal cutting temperature
OEtA	Oleoyl ethyl amide
PAK	P21-activated kinase
PAK	P21-Activated Kinase
PBS	Phosphate-buffered saline
PBS-T	Phosphate-buffered saline-Tween
PDBu	Phorbol-12,13-dibutyrate
PDE	Phosphodiesterase
PDE4Is	Phosphodiesterase 4 inhibitors
PDE5Is	Phosphodiesterase 5 inhibitors
PEDIs	Phosphodiesterase inhibitors
PG	Prostaglandin
PK	Protein kinases
PKC	Protein kinase C
PSA	Prostate-specific antigen
PTGS	Prostaglandin-endoperoxide synthase
PVDF	Polyvinylidene-Fluoride
PVR	Post-void residual
Qmax	Maximum flow rate
RAC	Ras-related C3 botulinum toxin substrate
Rho	Rhodopsin
RhoGEF	Rho guanine nucleotide exchange factor
RNA	Ribonucleic acid

ROCK	Rhodopsin kinase
RT	Reverse Transcription
RT-PCR	Real time polymerase chain reaction
SD	Standard deviation
SDS	Sodium dodecyl sulfate
SEM	Standard error of the mean
SFK	Src family kinase
TRPM8	Transient receptor potential melastin 8 ion channel
TURP	Transurethral resection of prostate
TXA2	Thromboxane
UR	Urinary retention
α1AR-blockers	α1-Adrenoceptor antagonists
β-3 agonists	Beta3 adrenoceptor agonists

11. Acknowledge

First, I would like to express my sincere gratitude to my supervisor, Prof. Dr. Christian Stief for offering me the opportunity to work and study in the Department of Urology in Klinikum Großhadern as a doctoral candidate, which greatly enhances my academic skills and also, provides me the opportunity to experience a precious period in Munich.

My very special gratitude goes to my co-supervisor, Priv. Doz. Dr. rer. nat. Martin Hennenberg for his consistent and reliable help for my daily life and research problems. His guidance always helps me a lot for my whole research, the writing of this thesis and my publications. It is extremely a wonderful research experience under the supervision by Dr. Hennenberg in the research lab of Urology.

I would like to express my deeply gratitude to Prof. Dr. med. Christian Gratzke for his kindness and precious opinions on my research work. My thanks also go to Priv. Doz. Dr. Frank Strittmatter, who is always ready to offer a helping hand for me, he shares to me many experiences both on research and surgery skills which are quite precious for me.

My thanks also go to Prof Dr E. Noessner and her co-workers for support with immunofluorescence microscopy. Also, I would to deeply thank Prof. Dr. Thomas Kirchner and his co-workers Dr. Vera Mai and Dr. Manuel Carranza, et al. from the Institute of Pathology, Ludwig Maximilian University, Munich for providing the tissue samples from prostates.

I would like to thank all the members in our lab, Dipl. Biol. Anna Ciotkowska,

Dipl. Chem. Beata Rutz, Dr. med. Annika Herlemann, Christian Sterr, Paul Kuppermann, Alexander Tamalunas, Ayse Nur Kan, Nicolas Nick, Dr. med. Yiming Wang, Xiaolong Wang, Bisheng Li for their help in my work and research.

I would like to thank the scholarship from China Scholarship Council (CSC) for financial support for my study and work in the Department of Urology, LMU.

Most importantly, I would like to deeply thank my family: my father Deming Yu, my mother Aijun Zheng, my younger sister Jia Yu, my wife Ning Li, and my dear son Mu Yu. They are always encouraging and supporting me, no words can express how much I love them and appreciate them, I owe them my rest life.

12. Curriculum vitae

NAME Qingfeng Yu

EDUCATION

2016-2018 Department of Urology, Klinikum Großhadern,
Ludwig-Maximilians-Universität München, Germany
Dr. med. candidate (Major: Urology)
Supervisor: Prof. Dr. med. Christian G. Stief
Co-supervisor: PD Dr. rer.nat Martin Hennenberg

2009-2012 Department of Urology, Nanfang Hospital,
Southern Medical University,
Master in Clinical Medicine (Major: Urology)
Supervisor: Prof. Ph.D Xiangming Mao.

2004-2009 The first clinical medical college,
Southern Medical University,
Bachelor in Clinical Medicine

WORK EXPERIENCE

2012-2015 Department of Urology,
Sun Yat-sen University Affiliated Shantou Hospital
Resident

RESEARCH EXPERIENCE

2009-2012 Laboratory of Clinical Medicine Research Center in Nanfang Hospital,
Southern Medical University
Direction of research: male infertility

2016-2018 Department of Urology of Klinikum Großhadern, Ludwig-MaximiliansUniversität München
Direction of research: New mediators of smooth muscle contraction in human prostate

PUBLICATIONS

Inhibition of prostatic smooth muscle contraction by the inhibitor of G protein-coupled receptor kinase 2/3, CMPD101.
Yu Q, Gratzke C, Wang Y, Herlemann A, Strittmatter F, Rutz B, Stief CG, Hennenberg M.
Eur J Pharmacol. [Accepeted]

Inhibition of human prostate smooth muscle contraction by the LIM kinase inhibitors, SR7826 and LIMKi3.
Yu Q, Gratzke C, Wang Y, Herlemann A, Sterr CM, Rutz, Ciotkowska A, Wang X, Strittmatter F, Stief CG, Hennenberg M.
Br J Pharmacol. 2018 Mar 25. doi: 10.1111/bph.14201. [Epub ahead of print]

Inhibition of smooth muscle contraction and ARF 6 activity by the inhibitor for cytohesin GEFs, secinH3 in the human prostate.
Herlemann A, Keller P, Schott M, Tamalunas A, Ciotkowska A, Rutz B, Wang Y, Yu Q, Waidelich R, Strittmatter F, Stief CG, Gratzke C, Hennenberg M.
Am J Physiol Renal Physiol. 2017 Aug 30:ajprenal.00125.2017 [Epub ahead of print]

Inhibition of agonist-induced smooth muscle contraction by picotamide in the male human lower urinary tract outflow region.
Hennenberg M, Tamalunas A, Wang Y, Keller P, Schott M, Strittmatter F, Herlemann A, Yu Q, Rutz B, Ciotkowska A, Stief CG, Gratzke C.
Eur J Pharmacol. 2017 May 15;803:39-47.

Non-Adrenergic, Tamsulosin-Insensitive Smooth Muscle Contraction is Sufficient to Replace α1 -Adrenergic Tension in the Human Prostate.
Hennenberg M, Acevedo A, Wiemer N, Kan A, Tamalunas A, Wang Y, Yu Q, Rutz B, Ciotkowska A, Herlemann A, Strittmatter F, Stief CG, Gratzke C.
Prostate. 2017 May;77(7):697-707.

Inhibition of Adrenergic and Non-Adrenergic Smooth Muscle Contraction in the Human Prostate by the Phosphodiesterase 10-Selective Inhibitor TC-E 5005.
Hennenberg M, Schott M, Kan A, Keller P, Tamalunas A, Ciotkowska A, Rutz B, Wang Y, Strittmatter F, Herlemann A, Yu Q, Stief CG, Gratzke C.
Prostate. 2016 Nov;76(15):1364-74.

Analysis of endothelial nitric oxide synthase (eNOS) G894T polymorphism and semen parameters in a Chinese Han population.
Yu Q, Zhang Y, Xia Y, Yang X, Li N, Ye L, Mao X.
Andrologia. 2014 Jun;46(5):541-6.

SEMG1 may be the candidate gene for idiopathic asthenozoospermia.
Yu Q, Zhou Q, Wei Q, Li J, Feng C, Mao X.
Andrologia. 2014 Mar;46(2):158-66.

HnRNPL as a key factor in spermatogenesis: Lesson from functional proteomic studies of azoospermia patients with sertoli cell only syndrome.
Li J1, Guo W, Li F, He J, Yu Q, Wu X, Li J, Mao X.
J Proteomics. 2012 Jun 6;75(10):2879-91.

13. Eidesstattliche Versicherung und Erklärung

Eidesstattliche Versicherung

Yu, Qingfeng

Name, Vorname

Ich erkläre hiermit an Eides statt,

dass ich die vorliegende Dissertation mit dem Thema
Inhibition of human prostate smooth muscle contraction by the LIM kinase inhibitors, SR7826 and LIMKi3.

selbständig verfasst, mich außer der angegebenen keiner weiteren Hilfsmittel bedient und alle Erkenntnisse, die aus dem Schrifttum ganz oder annähernd übernommen sind, als solche kenntlich gemacht und nach ihrer Herkunft unter Bezeichnung der Fundstelle einzeln nachgewiesen habe.

Ich erkläre des Weiteren, dass die hier vorgelegte Dissertation nicht in gleicher oder in ähnlicher Form bei einer anderen Stelle zur Erlangung eines akademischen Grades eingereicht wurde.

Munich, 24,11,2017

Ort, Datum

Qingfeng Yu

Unterschrift Doktorandin/Doktorand