CLONING OF *UBAP2* AND ITS ROLE IN PROSTATE CANCER

Master's thesis

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TIIVISTELMÄ

Tutkimuksen tausta ja tavoitteet: Eturauhassyöpä on yleinen syöpäsairaus, joka koskettaa maailmanlaajuisesti miljoonia miehiä. Sairauden taustalla olevat molekulaariset mekanismit ovat yhä huonosti tunnettuja. Genomin kopiolukumuutokset ovat yleisiä eturauhassyövässä, ja eräs toistuva monistuma sijaitsee kromosomissa 9p13.3. Tämän vastikään löydetyn monistuman tiedetään olevan yhteydessä korkeampaan PSA-arvoon sekä lyhyempään progressiovapaaseen elinaikaan prostatektomiapotilailla, mikä viittaa siihen, että monistuman alueella saattaa sijaita yksi tai useampia tuntemattomia onkogeeneja. *UBAP2* on eräs 9p13.3 monistuman potentiaalinen kohdegeeni. Tämän tutkimuksen tavoitteena oli kloonata *UBAP2*-geeni ja tutkia sen yli-ilmentymisen mahdollisia vaikutuksia eturauhassyöpäsolujen proliferaatioon.

Tutkimusmenetelmät: *UBAP2* kloonattiin TOPO-TA-kloonausmenetelmää käyttäen pCMV-Sport6-ilmentämisvektoriin. PC-3- ja LNCaP-eturauhassyöpäsoluja transfektoitiin väliaikaisesti *UBAP2*-geenin eri varianteilla ja solujen proliferaatiota tutkittiin AlamarBlue-määritysmenetelmällä sekä mikroskoopin ja digitaalikuva-analyysin avulla. *UBAP2*-lähetti-RNA:n yli-ilmentyminen varmistettiin kvantitatiivisella käänteiskopiointipolymeraasiketjureaktiolla, ja proteiinitason yli-ilmentyminen varmistettiin immunosytokemiallisesti.

Tutkimustulokset: Tutkimuksessa kloonattiin onnistuneesti täysimittainen *UBAP2*-geeni. Tämän lisäksi tutkimuksessa löydettiin useita ennestään tuntemattomia *UBAP2*-geenin silmikointivariantteja. Käänteiskopiointipolymeraasiketjureaktiosta saatujen tulosten perusteella *UBAP2*-geenillä transfektoidut eturauhassyöpäsolut ilmensivät *UBAP2*-lähetti-RNA:ta korkealla tasolla. Proteiinitasolla puolestaan osa transfektoiduista solupopulaation soluista ilmensi UBAP2-proteiinia korkealla tasolla, kun taas osa soluista ilmensi sitä hyvin vähän. Täysimittaisen *UBAP2*:n yli-ilmentäminen lisäsi hieman sekä PC-3- että LNCaP-solujen proliferaatiota, mutta vaikutus ei ollut tilastollisesti merkittävä.

Johtopäätökset: *UBAP2*-geenin transkriptioon ja silmikointiin liittyy paljon monimutkaisia mekanismeja. Vaikka tämän tutkimuksen tulosten perusteella *UBAP2*-geenin yli-ilmentämisellä ei ole tilastollisesti merkittävää vaikutusta eturauhassyöpäsolujen proliferaatioon, näiden tulosten perusteella ei voida täysin sulkea pois sitä mahdollisuutta, että *UBAP2* on 9p13.3-monistuman kohdegeeni. Lisätutkimuksia tarvitaan selvittämään *UBAP2*-geenin toimintaa sekä sen yli-ilmentämisen vaikutuksia eturauhassyövässä.

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ABSTRACT

Background and aims: Prostate cancer is a common malignancy affecting millions of men worldwide. The molecular mechanisms involved in prostate cancer are poorly understood. Genomic copy-number alterations are common in prostate cancer and one recurrently amplified region is 9p13.3. This recently identified amplification has been found to be associated with higher PSA-level and poor progression-free survival in prostatectomy treated patients, which indicates that the region harbors one or more novel oncogenes. *UBAP2* is one potential target gene of 9p13.3 gain. The aim of this study was to clone *UBAP2* gene and to investigate whether its overexpression would affect the proliferation of prostate cancer cells.

Methods: *UBAP2* gene was cloned with TOPO-TA cloning to pCMV-Sport6 expression vectors. PC-3 and LNCaP prostate cancer cells were transiently transfected with different *UBAP2* variants and their proliferation was assessed by AlamarBlue assay and by microscopy and digital image analysis. *UBAP2* mRNA overexpression was verified by using RT-qPCR analysis. Overexpression at the protein level was verified by using immunocytochemistry.

Results: In this study, the cDNA for full-length *UBAP2* gene was successfully cloned. In addition, we identified multiple novel splice variants of *UBAP2*. According to RT-qPCR results, prostate cancer cells transfected with *UBAP2* expressed its mRNA at high levels. At the protein level, a subset of the transfected cells expressed UBAP2 at a high level, while the others expressed it at a very low level only. Overexpression of the full-length *UBAP2* slightly increased the proliferation of PC-3 and LNCaP cells, but the differences were statistically insignificant.

Conclusion: There are many distinctive features associated with transcription and splicing of *UBAP2*. Although the results of this study suggest that *UBAP2* overexpression has no statistically significant effects on the proliferation of prostate cancer cells, we cannot rule out the possibility that *UBAP2* is the target gene of 9p13.3 amplification. Further investigations have to be carried out to determine the function of *UBAP2* and to clarify the role of its increased expression to prostate cancer.

CONTENTS

ABBREVIATIONS

aCGH	Array CGH
AKT	V-akt murine thymoma viral oncogene homolog
AR	Androgene receptor
ARL11	ADP-ribosylation factor-like 11
ATCC	American Type Culture Collection
BCAS3	Breast carcinoma amplified sequence 3
C9orf25	Chromosome 9 open reading frame 25
cCGH	Chromosomal CGH
CCND2	Cyclin D2
CCNL1	Cyclin L1
CD72	Cluster of differentiation 72
CDH13	Cadherin 13
CGH	Comparative genomic hybridization
CllD7	Chronic lymphocytic leukemia deletion gene 7
CMV	Cytomegalovirus
CRPC	Castration resistant prostate cancer
CYP1B1	Cytochrome P450, family 1, subfamily B, polypeptide 1
DCTN3	Dynactin subunit 3
ECT2	Epithelial cell transforming sequence 2 oncogene
ERBB2	v-erb-b2 erythroblastic leukemia viral oncogene homolog 2
ERG	Ets related gene
ESR1/2	Estrogen receptor 1/2
ETS	E twenty-six
ETV1/4/5	Ets variant 1/4/5
FISH	Fluorescence in situ hybridization
FKBP5	FK506 binding protein 5
GALT	Galactose-1-phosphate uridylyltransferase
GMPS	Guanine monophosphate synthetase
HER2	Human Epidermal Growth Factor Receptor 2
HERPUD1	homocysteine-inducible endoplasmic reticulum stress-inducible ubiquitin-like domain member 1
HGPIN	High-grade PIN
Hox	Homeobox
IGF2	Insulin-like growth factor 2
LB	Luria Broth
LINE1	Long interspersed nuclear element 1
MAF	v-maf musculoaponeurotic fibrosarcoma oncogene homolog
MLF1	Myeloid leukemia factor 1
MYC	v-myc myelocytomatosis viral oncogene homolog
NDRG1	N-myc downstream regulated 1
NGS	Next-generation sequencing
NKX3.1	NK3 homeobox 1
PC-3	PC-3 human prostate cancer cell line
PI3K	Phosphoinositide 3-kinase
PIGO	Phosphatidylinositol glycan anchor biosynthesis, class O

PIN	Prostatic intraepithelial neoplasia
PLAU	Plasminogen activator, urokinase
PSA	Prostate specific antigen
PTEN	Phosphatase and tensin homolog
PTES	Post transcriptional exon shuffling
RFP2	Ret finger protein 2
RGC32	Response gene to complement 32
RUSC2	RUN and SH3 domain containing 2
SKIL	SKI-like oncogene
SLC45A3	Solute carrier family 45 member 3
TBP	TATA-binding protein
TLOC1/SEC62	Translocation protein 1/ SEC62 homolog
TMPRSS2	Transmembrane protease, serine 2
TP53	Tumor protein 53
UBA	Ubiquitin-associated
UBAP2	Ubiquitin-associated protein 2
UBE2R2	Ubiquitin-conjugating enzyme E2R 2
UNC13B	Unc-13 homolog B
VCP	Valosin containing protein

1. INTRODUCTION

Prostate cancer is the second most common cancer of the men worldwide and the most common cancer among men living in western countries (Center et al. 2012). As the life expectancy is increasing in most countries and the prostate cancer incidence increases with the age, it is anticipated that the number of prostate cancer cases will continue to increase (Center et al. 2012). Worldwide, prostate cancer is the sixth leading cause of cancer-related death in men, although mortality is decreasing in many developed countries, mainly because of earlier diagnosis and improved treatment (Jemal et al. 2011).

Prostate cancer is a highly heterogeneous disease, both clinically and genetically (Boyd, Mao & Lu 2012). The majority of prostate tumors are slow-growing, metastasize rarely and they are not likely to cause death. However, some prostate cancers behave aggressively; they metastasize quickly and eventually progress to castration resistant prostate cancer (CRPC) with limited treatment options. One of the key questions is how to distinguish aggressive and indolent prostate cancers at an early stage. One strategy is to identify and detect biomarkers or genetic alterations that are associated with an aggressive disease. Some of these alterations are also potential therapeutic targets.

Prostate cancer is characterized by multiple genomic alterations, including both point mutations and chromosomal rearrangements (Boyd, Mao & Lu 2012). As prostate cancer is genetically a highly heterogeneous disease, patients typically harbor a diverse and unique set of alterations, which is affected also by geographic and ethnic factors. Generally, somatic point mutations seem to be rare relative to other cancers, while chromosomal copy number alterations are more frequently found (Taylor et al. 2010). Deletions in at least chromosomes 2q, 5q, 6q, 8p, 10q, 13q, 16q, 18q and 21q, and gains in chromosomes 3q, 7q, 8q, 9p, 17q and Xq are recurrently observed in prostate cancer (Cheng et al. 2012). These regions harbor many known cancer associated genes, and new genes are continuously identified.

Recurrent gain on chromosome 9p13.3 has previously been identified, and according to previous studies even 10% and 32% of prostatectomy treated patients harbor high-level

and low-level 9p13.3 amplifications, respectively (Leinonen 2007). In the same study, 9p13.3 gain was found to be associated with higher PSA-value and poor progression-free survival in prostatectomy treated patients (Leinonen 2007). Although the 9p13.3 region harbors multiple potential candidate genes whose expression is known to correlate with increased copy number, no cancer associated genes have been identified from this region (Leinonen 2007). Identification of the target gene of the 9p13.3 amplification might provide a new prognostic marker for prostate cancer and help to understand the molecular mechanisms underlying prostate cancer progression.

2. REVIEW OF LITERATURE

2.1 Prostate

Prostate gland is a part of male reproductive system. It accounts for the production and transportation of sperm and secretion of male sex hormones. The main function of the prostate is to store and secrete slightly alkaline, milky prostatic fluid. This fluid constitutes about half of the semen volume, along with spermatozoa and seminal vesicle fluid. Prostate gland is located below the bladder, as shown in the Figure 1.

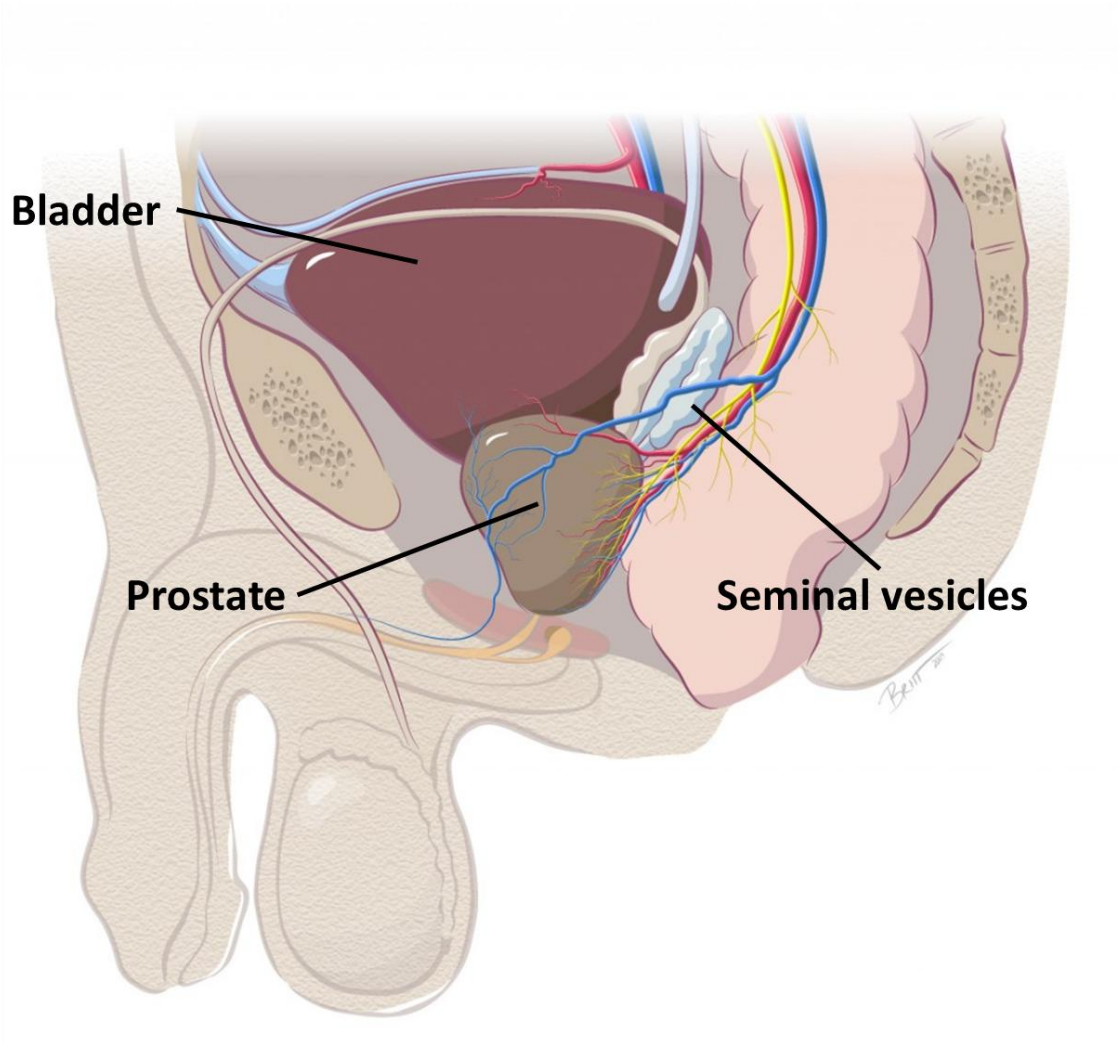

Figure 1. The prostate gland. The image shows the anatomical location of the prostate. Image modified from http://centegra.org/wp-content/uploads/page/Male_ Prostate_Anatomy_Sagital_View_-_low_res.jpg.

During the embryogenesis, the prostate is derived from the primitive endoderm, which first differentiates to cloaca. In humans, as well as in other placental mammals, the cloaca separates to digestive outlet and urogenital sinus, which then segments into the urinary bladder and the urethra. Prostate develops via the proliferation of epithelial buds from the urogenital sinus epithelium. These buds invade according to particular pattern, which directs the future development of distinct prostate zones (or lobes). Later, urogenital sinus mesenchymal cells differentiate into stromal elements. Prostate budding occurs during the 10^{th} week of embryogenesis. (Berman et al. 2012)

Androgen receptor (AR) signaling acts on the mesenchyme and is the primary motivating force behind prostate development (Berman et al. 2012). However, AR signaling only affects the timing of events, not their location. Mechanisms that direct the precise locations of prostate epithelial buds are not fully understood, but are likely to be related to paralogous homeobox (*Hox*) genes, as they coordinate similar processes in various tissues. It is also know that homozygous mutations in *Hox* genes can result in changes in prostatic branching patterns (Podlasek, Duboule & Bushman 1997).

The adult prostate is a complex tubule-alveolar gland, and the regional anatomy of the human prostate has been widely debated during the last century. Originally prostate was classified into lobes, but nowadays this concept is commonly replaced by zonal model (Timms 2008). According to this model, prostate is classified into anterior, peripheral, central and transitional zones that all have their own architectural features. The prostate is composed of epithelial compartment and stroma. The epithelial compartment consists of basal epithelial cells, intermediate cells, neuroendocrine cells and luminal secretory cells. The stromal compartment, in turn, serves a structural support consisting of connective tissue, smooth muscle cells and fibroblasts. (Berman et al. 2012)

While androgen signaling is important during development of the prostate in embryogenesis, it is also important for the growth, maintenance and secretory function of prostate during the different stages of human life.

2.1.1 Non-malignant prostate diseases

Prostatitis, inflammation of the prostate gland, is the most commons urologic problem of men younger than 50 years of age. Prostatitis can be caused by bacterial infection,

usually caused by *Escherichia coli*, and the infection can be either acute or chronic. Bacterial prostatitis is cured primarily by antibiotics. (Benway, Moon 2008) However, the vast majority, even 90%, of prostatitis cases are non-bacterial. This condition is characterized by pain or discomfort in the pelvic region, urinary symptoms and/or sexual dysfunction, and it can significantly diminish the quality of life. Although the etiology of non-bacterial prostatitis is unclear, it is known that factors like heredity background, infections, hormone imbalance, intraprostatic reflux, immunological or allergic triggers and psychological traits can incite it. (Anothaisintawee et al. 2011) The management of non-bacterial prostatitis is often quite complex, and applied treatments include α-blockers, antibiotics or anti-inflammatory medications (Thakkinstian et al. 2012). In addition to pharmacological treatments, other methods like prostatic massage, electromagnetic stimulation, physical therapy and thermotherapy are also used for the management of prostatitis (Schiller, Parikh 2011).

Benign prostatic hyperplasia (BPH) is a common condition in which the size of the prostate gland is increased. It is a common disease among ageing men, and it is associated with multiple bothersome lower urinary tract symptoms, such as frequent urination and the sensation of incomplete bladder emptying. BPH can be treated surgically or by drugs like α_1-adrenergic receptor blockers and 5-α-reductase inhibitors. Also other therapies are used. (Shrivastava, Gupta 2012) However, BPH does not give rise to a malign prostate disease.

BPH and prostate cancer form in different areas of the prostate; the former develops from the transitional zone or central zone of the gland, while the latter develops from the peripheral zone (De Nunzio et al. 2011). However, benign prostatic hyperplasia and prostate cancer share many significant anatomic, pathologic, genetic and epidemiological features. Many studies have focused on the relationship between BPH and the development of prostate cancer, but it is still unclear. (Alcaraz et al. 2009) It has been suggested that chronic prostatitis may be involved in the development and progression of both BPH and prostate cancer (De Nunzio et al. 2011).

2.2 Prostate cancer

2.2.1 Epidemiology

Prostate cancer is the most common malignancy and the third most common cause of death from cancer of men in the developed countries (Jemal et al. 2011). In Finland, almost 5000 new prostate cancer cases are diagnosed annually, and nearly 900 men die every year due to this cancer. Hence, prostate cancer is the second most common cause of death from cancer of men in Finland. Prostate cancer incidence has increased during the past decades, but at the same time prostate cancer specific mortality has stayed relatively stable. (Finnish Cancer Registry, 2012). Similar trends can be seen in other Western countries as well (Center et al. 2012, Brawley 2012, Crawford 2003).

The increase of the prostate cancer incidence in developed countries mainly results from increased life expectancy, advanced diagnostic methods and the generalization of PSA screenings (Center et al. 2012). Prostate cancer affects mostly old men; over 70% of prostate cancer cases are diagnosed in men over 65 years of age, and the disease is only relatively rarely diagnosed in men less than 50 years of age (Crawford 2003). As the average life expectancy in many developing countries is less than 65 years, it is easy to understand why prostate cancer is not as common in developing as in developed countries (WHO World Health Statistics 2012).

2.2.2 Pathogenesis

Prostate cancer arises from glandular epithelium, most commonly from the peripheral zone. At first, there will appear prostatic intraepithelial neoplasia (PIN) lesions that are progressive abnormalities with genotypes and phenotypes that are an intermediate between those of benign prostatic epithelium and prostate cancer. PIN lesions are characterized by the proliferation of secretory cells with significant cytological abnormalities within the prostate glands and acini. These cells typically have an increased nuclear/cytoplasmic ratio and prominent nucleoli. In contrast to prostate adenocarcinoma, in PIN lesions the basal cell layer is retained, while it is absent in cancer. (Bostwick, Cheng 2012, Klink et al. 2012) PIN lesions are classified into low grade (LGPIN) and high grade (HGPIN) lesions according to cytological characteristics (Goeman et al. 2003). The hypothesis that HGPIN is a precursor of prostate cancer is

widely accepted and a lot of evidence supporting it has been presented. The incidence of both HGPIN and prostate cancer increase with age, and the frequency and the severity of HGPIN increases in the presence of prostate cancer. HGPIN shares many features with prostate cancer and the transition of HGPIN to prostate cancer can be observed morphologically. Moreover, both HGPIN and prostate cancer have similar genetic and molecular features. (Montironi et al. 2007)

PIN lesions are a common finding in prostate biopsies; the total incidence of isolated PIN averages 9% in United States, which represents about 115 000 new cases each year (Bostwick, Cheng 2012). The frequency of cancer detection on repeat biopsy after diagnosis of HGPIN is about 20-30% (Schoenfield et al. 2007, Gallo et al. 2008, Roscigno et al. 2004, Epstein, Herawi 2006, Herawi et al. 2006). This frequency is not far from the frequency of cancer detection after diagnosis of benign prostatic hyperplasia, which is about 20% (Montironi et al. 2007, Gallo et al. 2008). In studies published before the year 2000, cancer detection rate following HGPIN diagnosis was even 36%. The decrease in the percentage may result from increased biopsy core number routinely sampled, as previously HGPIN findings on needle biopsies were often representing sampling problems with carcinomas nearby. (Klink et al. 2012) However, the predictive value of isolated HGPIN can be higher in two situations; if HGPIN is adjacent to atypical glands or in case of multifocal HGPIN (Schoenfield et al. 2007, Roscigno et al. 2004).

2.2.3 Risk factors

Age, race and positive family history are the most important prostate cancer risk factors. Also some other factors, like over-weight, smoking, taller height and high α-linolenic acid intake have been found to be associated with high prostate cancer risk (Giovannucci et al. 2007). In United States, prostate cancer incidence rates have significantly increased among men younger than 50 years old during the last decade. At the same time the incidence rates among men older than 70 years have decreased, which suggests that these changes result from earlier diagnosis. (Li et al. 2012)

A family history of prostate cancer is known to be associated with increased risk of prostate cancer diagnosis (Thomas et al. 2012). According to some studies, first-degree relatives of prostate cancer patients have a 2,5-fold relative risk of prostate cancer,

while first-degree relatives of two prostate cancer patients have a 3,5-fold relative risk. The risk is even higher, if prostate cancer was diagnosed before the age 60, and it decreases with age (Johns, Houlston 2003). It has also been found out that men with first-degree relatives affected by prostate cancer are diagnosed and die at earlier age than men without relatives affected by prostate cancer (Brandt et al. 2009).

African American males have over 2-fold risk of prostate cancer and also have over 2-fold prostate cancer specific mortality when compared to Caucasian males in the United States (American Cancer Society 2012). The reason for this is poorly understood, but lower income among the African American population may affect the higher mortality (Taksler, Keating & Cutler 2012).

According to a large recent cohort study, current smokers have an increased risk of fatal prostate cancer, but a decreased risk of non-advanced prostate cancer (Watters et al. 2009). Similarly, former smoking was associated with decreased risk of non-advanced prostate cancer. However, the association shown in this study were not very strong. (Watters et al. 2009). In a prospective observational study within North American prostate cancer patients, it was observed that smoking at the time of cancer diagnosis was associated with increased overall mortality and cardiovascular disease and prostate cancer specific mortality and cancer recurrence (Kenfield et al. 2011). It seems that smoking does not actually increase the risk of prostate cancer diagnosis, but it seems to increase the probability to dying due to prostate cancer.

Alcohol consumption is known to be a risk factor of numerous cancers. In a large cohort study within the North American population, the risk of non-advanced prostate cancer was 25% higher for men consuming at least 6 servings of alcohol daily, 19% higher for men consuming 3-6 serving of alcohol daily and 6% higher for men consuming up to 3 serving daily, when comparing to nondrinkers (Watters et al. 2010). However, an association between advanced prostate cancer and alcohol consumption was not observed. On the contrary, in another study within New Zealand cohort, alcohol consumption was associated with a reduced risk of prostate cancer (Karunasinghe et al. 2013). Meta-analysis of 50 case-control and 22 cohort studies, in turn, provided no evidence of association between alcohol consumption and prostate cancer risk (Rota et al. 2012).

Recently, high coffee consumption was observed to reduce the risk of advanced prostate cancer (Wilson et al. 2011). However, according to another cohort study, there is no association between coffee consumption and overall prostate cancer risk (Shafique et al. 2012). The association of α-linolenic acid, which is the most common omega-3 fatty acid in the Western diet, and prostate cancer risk has also been explored in many studies, but so far the results have been conflicting (Koralek et al. 2006, De Stefani et al. 2000, Brouwer, Katan & Zock 2004, Simon, Chen & Bent 2009). These studies have mainly explored the association of dietary α-linolenic acid consumption to prostate cancer. According a recent study, prostatic α-linolenic acid, independent of the amount of α-linolenic acid consumed, is associated with prostate cancer (Azrad et al. 2012). This could explain the conflicting results of the previous studies.

In addition to multiple other health problems, obesity is known to increase the risk of prostate cancer. Although obesity seems to even decrease risk of non-advanced prostate cancer, it significantly increases the risk of advanced prostate cancer and prostate cancer specific mortality (Rodriguez et al. 2001b, Rodriguez et al. 2001a, Engeland, Tretli & Bjorge 2003, Wright et al. 2007, Andersson et al. 1997, Rodriguez et al. 2007). Obesity also increases the risk of prostate cancer progression to a castration resistant prostate cancer after androgen deprivation therapy (Keto et al. 2012). The reason for the association of obesity and prostate cancer is not fully understood, but it is known that obesity affects sex hormone levels (Keto et al. 2012). In addition to body weight, also height has been found to be associated with prostate cancer risk. Studies carried out among Norwegian, Japanese and North-American populations have pointed out that height is a significant prostate cancer risk factor; taller men have higher prostate cancer incidence and mortality than shorter men (Rodriguez et al. 2001a, Engeland, Tretli & Bjorge 2003, Minami et al. 2008). The association between height and higher prostate cancer risk could be explained by differences in the level of insulin-like growth factor. Tall men have higher levels of insulin-like growth factor, and increased level of this growth factor has been shown to be associated with prostate cancer (Chan et al. 1998, Stattin et al. 2000, Gunnell et al. 2001).

2.3 Prostate cancer genetics

Prostate cancer is clinically a highly heterogenic disease. There can be many tumor foci in the same prostate gland, and these foci can represent different histological degrees. Also tumors representing same histological degrees may lead to different clinical outcomes. Genetic heterogeneity can explain this heterogeneity, at least to some extent. In turn, genetic heterogeneity results from genomic instability. (Boyd, Mao & Lu 2012) Despite the heterogenic nature of prostate cancer, recent studies suggest that on contrary to what was previously thought, the majority of multifocal prostate cancers may have monoclonal origins (Boyd et al. 2012, Lindberg et al. 2013).

Heredity plays an important role also in prostate cancer. It has been estimated that autosomal dominant inheritance causes 5 – 10% of all prostate cancer cases, and a vast majority of early onset diseases (Bratt 2002, Lange et al. 2012). Germ-line mutations in high-penetrance susceptibility genes are characteristic in hereditary prostate cancer (Alberti 2010). For instance, *RNASEL*, *ELAC2* and *MSR1* are genes frequently harboring mutations in hereditary prostate cancer. Furthermore, somatic mutations in these genes are associated with sporadic prostate cancer (Noonan-Wheeler et al. 2006). On the contrary to the small number of known high-penetrance prostate cancer susceptibility genes, there are a large number of low-penetrance susceptibility genes. The interaction of environmental factors and alterations in multiple low-penetrance susceptibility genes plays a major role in the vast majority of prostate cancer cases. Moreover, a positive family history is the most important risk factor also in the case of non-hereditary prostate cancer. (Boyd, Mao & Lu 2012)

As new technologies, such as comparative genomic hybridization (CGH) and next-generation sequencing (NGS), are developed and evolve, knowledge about the genetic and genomic alterations in cancer is enormously increased. Along this, it becomes more and more clear that the genetic basis of prostate cancer is complex and diverse. Any single alteration is not enough to cause prostate cancer. It is commonly believed that cancer results from the accumulation of a large number of mutations in the same cell, also known as somatic evolution. Mutations are often classified to the driver and passenger mutations. Driver mutations are alterations that confer a selective growth advantage to the cell, thus driving it toward the development of cancer. On the contrary,

passenger mutations have no impact on the growth of the cell, but they occur in a cell subsequently or coincidentally with driver mutations. According to some studies, human solid tumors typically contain 40-100 coding gene alterations, including 5-15 driver mutations. (Bozic et al. 2010) In addition, along the development and progression of cancer, genetic instability of the cells increases, meaning that they accumulate genetic changes at an abnormally rapid rate (Alberts et al. 2008).

2.3.1 Chromosomal gains and deletions

In comparison with other cancers, prostate cancer harbors relatively few protein-altering point mutations. Instead, chromosomal gains and deletions and gene fusions are more common, and multiple copy-number alterations are known to occur in prostate cancer (Barbieri, Demichelis & Rubin 2012)(Cheng et al. 2012). Frequent losses have been identified in at least the chromosomes 2q, 5q, 6q, 8p, 10q, 13q, 16q, 18q and 21q. Gains have been identified in the chromosomes 3q, 7q, 8q, 9p, 17q and Xq.

Alterations in the chromosome 8 are among the most common alterations in prostate cancer. The association of 8q gain and 8p deletion with prostate cancer progression has been known for nearly two decades (Matsuyama et al. 1994, Van Den Berg et al. 1995). Along the development of new methods, it has been possible to map at least three independent 8q regions that are recurrently amplified in prostate cancer (Saramaki et al. 2006). The prevalence of these amplifications increases among the progression of prostate cancer; in one study 8q gain was found from 5% of local prostate cancers, but from nearly 60% of hormone refractory prostate cancers. The same is applies also for 8p deletion (El Gammal et al. 2010). There are probably multiple genes affecting cancer progression in chromosome 8. Notable genes of this chromosome include the well-known oncogene *MYC* which locates on 8q24 and known prostate tumor suppressor *NKX3.1* locating on 8p21 (Boyd, Mao & Lu 2012).

Another remarkable chromosomal alteration in prostate cancer is the gain of chromosome Xq. The gene encoding androgene receptor (AR) locates on Xq12, and this locus has been found to be amplified in about 30% of CRPC cases, but nearly never in the early stages of cancer (Visakorpi et al. 1995). This amplification leads to AR overexpression, which can sensitize prostate cancer cells to androgen and makes androgen deprivation therapy inefficient (Waltering et al. 2009).

Deletion of 10q is yet another remarkable alteration in prostate cancer, since a commonly known tumor suppressor, *phosphatase and tensin homolog* (*PTEN*) gene locates on 10q23. Deletion of 10q is found from around 10% of prostate cancers (Sun et al. 2007). The loss of PTEN in prostate cancer correlates with an advanced stage of cancer and poor prognosis (McMenamin et al. 1999). In addition to prostate cancer, 10q deletion is also commonly found in bladder and breast cancers and in gliomas (Cairns et al. 1998, Li et al. 1997). In addition, PTEN point-mutations have been found from some inherited cancers and diseases (Lynch et al. 1997).

17q gain is one of the most common copy-number alterations in prostate cancer and it is found from about 65% of primary prostate cancers (Bermudo et al. 2010). Similar amplification is common also in some other cancers, such as neuroblastoma and breast cancer (Bown et al. 2001, Barlund et al. 2002). *MAFG*, which belongs to the family of MAF proto-oncogenes and *BCAS3*, which is suggested to have a role in breast cancer development, locate in the 17q gain region (Kannan, Solovieva & Blank 2012, Gururaj et al. 2006).

Similarly to prostate cancer, 13q deletion is a common alteration in many other cancer, such as leukemia, lung cancer, head and neck cancer and esophageal cancer (Zhou, Munger 2010). 13q contains several tumor suppressor genes, like *ARL11*, *RGC32*, *RFP2* and *CllD7* (Zhou, Munger 2010). On chromosome 3q, several areas have been found to be amplified often in prostate cancer (Sattler et al. 2000, Strohmeyer et al. 2004). These loci contain many potential oncogenes, such as *TLOC1/SEC62* (Jung et al. 2006), *GMPS*, *MLF1*, *SKIL*, *CCNL1* and *ECT2* (Sun et al. 2007).

2.3.1.1 9p13.3 gain

9p13.3 gain is a recently found recurrent gain in prostate cancer. Saramäki et al. screened 13 xenografts and 5 prostate cancer cell lines by using array comparative genomic hybridization (aCGH) method. As a result, they identified a novel chromosomal gain at 9p13-q21 (Saramaki et al. 2006). The minimal region of the gain was mapped to 9p13.3. 9p13-q21 gain had been previously detected in DU145 cell line by cCGH, but proper analysis of this region had been lacking, as regions close to centromeres cannot be reliably analyzed by cCGH (Nupponen et al. 1998). Also Taylor et al. detected 9p13.3 gain from multiple prostatectomy samples (Taylor et al. 2010). In

yet another study, 9p13-q21gain was found from one prostate cancer metastasis sample and 9p12-13 from another sample by using aCGH method (Paris et al. 2003).

In later studies, a high-level amplification of 9p13.3 was found to occur at 10% of prostatectomy specimens, while a low-level amplification was found from 32% of the samples. In addition, this amplification was associated with a higher PSA-level and poor progression-free survival in prostatectomy-treated patients (Leinonen 2007). In the same study, association between copy numbers and expression levels of the genes locating in 9p13.3 was studied by using RT-qPCR and FISH methods. According this study, promising candidate target genes for the 9p13.3 gain were *PIGO*, *UNC13B*, *CD72*, *VCP*, *GALT*, *DCTN3*, *C9orf25*, *UBAP2*, *RUSC2* and *UBE2R2* (Leinonen 2007). Moreover, also Taylor et al. analyzed gene expression levels and copy numbers within the 9p13.3 gain, and they found out that the expression of *UBAP1*, *UBAP2*, *UBE2R2* and *WDR40A* correlated with the gains in copy-numbers (Taylor et al. 2010).

2.3.2 Gene fusions

In general, there are two different gene fusion mechanisms that both can lead to tumorigenic alterations. Chromosomal rearrangements can promote the formation of chimeric fusion genes, which results in the expression of fusion proteins with altered activities. On the other hand, a gene can end up under the control of a different promoter, which leads to its altered expression (Boyd, Mao & Lu 2012). Previously, chromosomal rearrangements and gene fusions were thought to be common mainly in haematological malignancies and sarcomas (Kumar-Sinha, Tomlins & Chinnaiyan 2008). However, technical limitations have hindered searching and studying gene fusions from solid malignancies. Recent advances in genomic profiling and bioinformatics, for example, have revealed gene fusions also from other cancers, including prostate cancer (Boyd, Mao & Lu 2012).

The most common gene fusion in prostate cancer is *TMPRSS2-ERG* fusion. This fusion, found by Tomlins et al. in 2005, is found roughly from half of all prostate cancers (Boyd, Mao & Lu 2012, Tomlins et al. 2005). The fusion of *ERG*, a member of the ETS transcription factor family, with the promoter region of androgen-regulated *TMPRSS2* gene leads to the formation of an androgen-responsive oncogene (Rubin, Maher & Chinnaiyan 2011). The mechanism of this fusion is usually internal deletion or

chromosomal translocation. The results of studies focusing on the association between the presence of *TMPRSS2-ERG* fusion gene and the clinical outcome of prostate cancer have been slightly conflicting, but the fusion is possibly associated with poor clinical outcome (Boyd, Mao & Lu 2012).

Instead of *ERG*, *TMPRSS2* is sometimes fused with the other members of the ETS family, like *ETV1*, *ETV4*, *ETV5* or *ELK4* (Tomlins et al. 2005, Rubin, Maher & Chinnaiyan 2011). Also gene fusions of ETS transcription factors with 5'-fusion partners other than *TMPRSS2*, such as *SLC45A3*, *NDRG1*, *HERPUD1* and *FKBP5* have been reported (Boyd, Mao & Lu 2012). However, they are much more infrequent events than the *TMPRSS2-ERG* fusion and their significance for the progression and clinical outcome of prostate cancer is unclear.

2.3.3 Epigenetics

Epigenetic changes do not permanently alter the DNA sequence of genes. Instead, they induce conformational changes in the DNA double helix, thus affecting the access of transcriptional factors to promoter regions or to other regulatory sequences. Epigenetic changes can be preserved from cell to cell and they can also be heritable. (Albany et al. 2011) Epigenetic regulation is very important for the development of eukaryotic organisms, but it also plays a role in many human diseases and dysfunctions.

Like in many other cancers, epigenetic changes have a role also in prostate cancer. DNA methylation and histone modifications are the two best understood epigenetic mechanisms and they both are known to affect prostate cancer growth and metastasis. Hypermethylation of CpG rich areas in the promoter regions of several genes occurs frequently in prostate cancer (Paone, Galli & Fabbri 2011). This results in transcriptional silencing of the genes under the control of the hypermethylated promoter regions. The promoter regions of many tumor suppressor genes are known to be frequently hypermethlylated in prostate cancer, and silencing of these genes promotes a carcinogenetic effect (Paone, Galli & Fabbri 2011). Genes frequently hypermethylated in prostate cancer include for example *GSTP1, APC* and *RAFFS1a* (Jeronimo et al. 2011). On the contrary to hypermethylation, hypomethylation of promoter regions leads to increased expression of the genes regulated by these promoters. When compared to hypermethylation, promoter hypomethylation is far more infrequent in prostate cancer,

although it is known to be an important mechanism in some other cancers (Jeronimo et al. 2011). However, some genes, including *LINE1*, *IGF2*, *PLAU* and *CYP1B1*, have been reported to be hypomethylated also in prostate cancer (Jeronimo et al. 2011).

MicroRNAs (miRNAs) are small non-coding RNAs that have complex regulatory functions in cells. miRNAs act mainly by binding to the 3'-untranslated region of their target mRNA thus silencing them. However, they can also promote translation by binding to the 5'-untranslated region of their target transcripts (Paone, Galli & Fabbri 2011). It is also known that miRNAs can affect the epigenetinc control of genes. In cancer cells, this can lead to reduced methylation and re-expression of previously epigenetically silenced tumor suppressor genes (Paone, Galli & Fabbri 2011). In addition, genes coding miRNAs can undergo the same epigenetic regulatory mechanism discussed above, and hence, they can be silenced similarly to protein coding genes (Jeronimo et al. 2011). The interplay of miRNA induced translational silencing and epigenetic mechanisms creates a complex network of regulatory events, which is not yet fully understood. However, this miRNA-epigenetics network seems to have a significant role in many disorders, including prostate cancer.

2.3.4 Oncogenes

Genes, in which a gain-of-function mutation can contribute to cancer, are called proto-oncogenes (Alberts et al. 2008). Proto-oncogenes can become active oncogenes either through point-mutations, amplifications or rearrangements (Alberts et al. 2008). Many oncogenes have a fundamental impact on signaling pathways and they are critical in various types of cancer, while some oncogenes are specifically active in only certain types of cancers (Alberts et al. 2008).

MYC is a regulatory gene that encodes transcription factor MYC, which is thought to regulate 15 % of all human genes (Dasgupta, Srinidhi & Vishwanatha 2012). MYC is involved in the regulation of cellular proliferation, differentiation and apoptosis, (Grandori et al. 2000). *MYC* is also known to act as an oncogene in various cancers, frequently through genomic amplification (Dang 2012). As previously mentioned, *MYC* is located in the chromosomal region 8q24, which is recurrently amplified in prostate cancer (Boyd, Mao & Lu 2012).

In contrast to *MYC*, *AR*, which encodes androgen receptor, can be seen as a cancer-specific proto-oncogene. Androgen receptor is a nuclear receptor that has many functions in human development and physiology. On the other hand, androgen receptor is also involved in all stages of prostate tumorigenesis (Li, Al-Azzawi 2009). *AR* often transforms to oncogene during prostate cancer progression (Visakorpi et al. 1995, Han et al. 2005). This most commonly happens through gene amplifications, while gain-of-function mutations in AR are very rare (Visakorpi et al. 1995, Hay, McEwan 2012). Interestingly, androgen receptor may occasionally have an oncogenic role also in breast cancer. (Hickey et al. 2012).

ERBB2 is a gene that encodes HER2, which is a member of the epidermal growth factor receptor family. *ERBB2* is located in the chromosomal region 17q12, which is often amplified in prostate cancer, as well as in breast cancer and in some other malignancies. In cells, HER2 receptor acts by inducing the activation of the PI3K/Akt signaling pathway. Uncontrolled activation of this central signaling pathway leads to tumorigenic events in cells (Le Page et al. 2012). The overexpression of *ERBB2* has a high clinical relevance in the treatment of breast cancer, as cancers overexpressing it can be treated by monoclonal antibodies against HER2 (Carter et al. 1992). In the case of prostate cancer, however, the expression of *ERBB2* is relatively low it is not a relevant target for cancer treatments (Savinainen et al. 2002).

2.3.5 Tumor suppressor genes

In contrast to oncogenes, tumor suppressor genes are genes in which a loss-of-function mutation can drive a cell towards the development of cancer (Alberts et al. 2008). Tumor suppressor genes can be inactivated in many ways, for example by chromosomal deletions, point mutations or by epigenetic mechanisms. Unlike with oncogenes, usually both alleles of a tumor suppressor gene have to be altered for cancer to develop (Alberts et al. 2008). Typically tumor suppressor genes regulate either the detection and repair of DNA damage, protein ubiquitination and degradation or cell cycle checkpoint responses (Sherr 2004).

Loss-of-function mutations in *TP53*, the gene coding p53 protein, are common in prostate cancer, as well in many other cancers (Isaacs, Kainu 2001). p53 is activated by phosphorylation in response to cellular stress such as DNA damage, and when activated

it acts as a transcription factor. The activation of the p53 pathway can result in cell cycle arrest, cell senescence and apoptosis. (Levine 2011) A recent study demonstrated that docetaxel, a drug that is commonly used as the first line treatment for castration resistant prostate cancer, induces the phosphorylation of p53. In accordance with this finding, the mutational status of p53 is determinant of docetaxel sensitivity of prostate cancer cells (Liu et al. 2013).

PTEN is yet another well-known tumor suppressor gene. It is commonly inactivated in many different types of cancer, such as glioma, melanoma and carcinoma of the endometrium, kidney, breast, lung, upper respiratory track and prostate cancers (Li et al. 1997, Pourmand et al. 2007). *PTEN*, located on 10q23, encodes a dual-specificity phosphatase and is known to act as a part of the PI3K-PTEN-AKT signaling pathway. This pathway is important in the regulation of multiple essential cellular processes like apoptosis, cell metabolism, cell proliferation and cell growth (Pourmand et al. 2007, de Muga et al. 2010). Inactivation of *PTEN* and the subsequent activation of the AKT pathway is a frequent event in prostate cancer progression, and the lack of *PTEN* expression is known to correlate with advanced pathological state and with a high Gleason score (de Muga et al. 2010). The frequency of *PTEN* mutations in metastatic prostate cancer varies among the different studies, but usually it is reported to be between 20 and 60 % (Pourmand et al. 2007, de Muga et al. 2010).

3. AIMS OF THE RESEARCH

The goal of this Master's thesis study was to clarify the role of *UBAP2* in prostate cancer. Previous studies have suggested that *UBAP2* might be a potential oncogene and a target gene of chromosomal gain in 9p13.3. The main aims of the study were:

1) Cloning *UBAP2* gene.
2) Analyzing the effects of overexpression of *UBAP2* in prostate cancer cells.

4. MATERIAL AND METHODS

4.1 Cloning of *UBAP2*

The starting material for the cloning of *UBAP2* was commercial Human Universal Reference RNA (Agilent) and RNA previously extracted from PC-3, DU145 and 22Rv1 cell lines. cDNA was synthesized from extracted RNA by using SuperScript III reverse transcriptase (Invitrogen) together with oligo(dT) primers (Invitrogen). The cDNA was used as the template for PCR amplification of the UBAP2 gene. The primers used in this PCR are shown in the table 1., and they are complementary to the first and the last exons of *UBAP2*. The PCR was performed using Phusion™ High-Fidelity DNA Polymerase (Finnzymes) under the default conditions recommended by the manufacturer. PCR products were purified in 1% agarose and visualized by ethidium bromide. The amplified DNA was extracted from the agarose gel by using QIAquick Gel Extraction Kit (Qiagen). As Phusion™ High-Fidelity DNA Polymerase generates blunt-ended products, 3' A-overhangs were added to the purified PCR products by using Dynazyme™ II DNA Polymerase (Finnzymes) and adenosine nucleotides. The reaction was incubated with the recommended buffer at 72 °C for 20 minutes.

The cloning of *UBAP2* was performed by using TOPO-TA based TOPO-XL cloning kit (Invitrogen) and pCR-XL-TOPO® vector (Invitrogen). The constructs were then transformed into One Shot® chemically competent TOP10 *E. coli* cells (Invitrogen) using heat-shock transformation. Cells were cultured overnight on LB plates containing 50 µg/ml of kanamycin. Transformation was performed according to manufacturer's instructions. To ensure successful cloning, colony PCR was performed on multiple colonies with *UBAP2* specific primers. Primers used for the colony PCR were same as used for RT-qPCR. The sequences of these primers are shown in the table 1. The products of the colony PCR products were loaded onto 1% agarose gel and visualized by ethidium bromide. Positive colonies were cultured overnight in small volumes of LB medium containing 50 µg/ml of kanamycin. Plasmid DNA was extracted by using GenElute™ Plasmid Miniprep Kit (Sigma-Aldrich).

4.2. DNA sequencing

Plasmid DNA was sequenced by Sanger's method using BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems) and ABI-3130xl genetic analyzer (Applied Biosystems). DNA was amplified prior to sequencing using Bio-Rad C1000^{TM} Thermal Cycler (Bio-Rad). Each sequencing reaction included 100-300 ng of DNA, 6 pmol of primers, 1,5 µl of 5x sequencing buffer and 1,0 µl of BigDye Terminator reaction mix (Applied Biosystems). The volume of the sequencing reactions were adjusted to 10 µl using sterile, deionized water. Primers used for the sequencing PCR are shown in the table 1. The sequencing reactions were denatured at 96 °C for 1 minute, followed by 30 cycles of 10 s at 96 °C, 10 s at 50 °C and 4 min at 60 °C. Amplified DNA was precipitated by adding 25 µl of 96% ethanol and 1 µl of 3 M sodium acetate (pH 5.2) and incubating the reactions for 15 minutes at room temperature. Precipitated DNA was pelleted by centrifugation at 2000 g for 45 minutes, and supernatants were discarded. The DNA pellets were washed with 125 µl of 70% ethanol and pelleted again by centrifugation at 2000 g for 15 minutes. Supernatants were discarded, the DNA pellets were air-dried at room temperature and resuspended into 12,5 µl of Hi-Di^{TM} formamide (Applied Biosystems). Resuspended DNA was denatured by incubating the samples at 95 °C for 3 minutes, followed by cooling on ice. Sequencing was performed by using ABI 3130xl genetic analyzer (Applied Biosystems). Sequences were analyzed with Chromas Lite 2.1 software (Technelysium).

4.3. Subcloning to expression vector

The *UBAP2* inserts confirmed by sequencing were subcloned into the pCMV-SPORT6 expression plasmid by using MluI and NotI restriction enzymes. Restriction enzymes, T4-DNA ligase and the used buffers were purchased from New England Biolabs. Restriction reactions were performed by double digestion in 1x NEBuffer 3 at 37 °C for 30 minutes. After restriction, *UBAP2* inserts and linearized pCR-XL-TOPO® plasmids were separated on 1% agarose gel. The UBAP2 inserts were purified using QIAquick Gel Extraction Kit (Qiagen). Linearized pCMV-SPORT6 plasmid was purified using QIAquick PCR Purification Kit (Qiagen). The ligation reactions of linearized pCMV-SPORT6 plasmid and *UBAP2* inserts were performed at room temperature for 10 minutes. Constructs were transformed into One Shot® chemically competent TOP10

e. coli cells. Transformed cells were then cultured overnight on LB plates containing 50 µg/ml of ampicillin. Colony PCR was performed on multiple colonies with *UBAP2* specific primers as described above. Positive colonies were cultured overnight in small volumes of LB medium containing 50 µg/ml of ampicillin. Plasmid DNA was extracted by using GenElute™ Plasmid Miniprep Kit (Sigma-Aldrich). DNA was sequenced as previously described. The constructs that were selected for overexpression experiments were cultured overnight in larger volumes of LB medium containing 50 µg/ml of ampicillin. After an overnight incubation at 37 °C, plasmid DNA was extracted by using Qiagen Maxiprep kit (Qiagen).

4.4 Cell lines

Both prostate cancer cell lines used in this study, PC-3 and LNCaP, were obtained from American Type Culture Collection (ATCC). The cells were cultured at 37 °C and 5% CO_2. Cell cultures were subcultured every three to four days. The basal media used were Ham's F12 with 10% fetal bovine serum and 2 mM L-glutamine for PC-3 cell line and RPMI 1640 with 10% fetal bovine serum and 2 mM L-glutamine for LNCaP cell line. Basal media and fetal bovine serum were purchased from Lonza.

4.5 Transient transfection

Transfection of prostate cancer cells with *UBAP2* constructs were performed using jetPEI® transfection reagent (Polyplus-transfection). 15 000, 20 000 or 30 000 cells were seeded on 24-well plate and incubated 24h before transfection. Transfections were performed according to manufacturer's instructions, using 500 ng of DNA per 1 cm2 area. Equal mass amounts of different *UBAP2* constructs were transfected to cells, and control cells were transfected with expression vectors lacking the *UBAP2* insert.

4.6 Proliferation assay

Cells were quantified by using AlamarBlue assay (Invitrogen) and light microscopy one, three and five days after transfection. Six parallel samples were used in each experiment. For AlamarBlue assay, 50 µl of AlamarBlue reagent was added to 1 ml of medium and 100 µl of medium was collected 90 minutes after adding the AlamarBlue. Absorbances of collected samples were then measured spectrophotometrically at

570 nm using the 2104 EnVision® Multilabel Reader (PerkinElmer). In this assay, increase or decrease in the metabolic activity of cell culture is measured by assaying the relative absorbances of the samples. In turn, the overall metabolic activity of the cell culture generally correlates with the number of cells in the culture. The number of cells was also quantified by imaging them using Olympus IX71 inverted light microscope (Olympus) and Surveyor software (Objective Imaging). Images were analyzed and cell surface areas were determined by using ImageJ software (National Institutes of Health).

4.7 RNA extraction and RT-qPCR

Total RNA was extracted from transfected cells using TRI Reagent® (Sigma). RNA extraction was performed according to manufacturer's instructions. For extraction, 200 000 cells were seeded on 6-well plate and transfected with *UBAP2* or control constructs one day prior to RNA extraction as previously described.

For real time quantitative PCR (RT-qPCR), RNA was reverse transcribed with SuperScript III reverse transcriptase (Invitrogen) by using oligo(dT) primers (Invitrogen). Standard curves were prepared from RNA extracted from non-transfected PC-3 cells by using 5-fold dilution series. All expression values were normalized to housekeeping gene *TBP* (TATA binding protein) and sterile water was used as negative control. RT-qPCRs were carried out in Bio-Rad CFX96™ Real-Time PCR Detection System (Bio-Rad) with Maxima^{TM} SYBR Green/ROX qPCR Master Mix (Fermentas). Manufacturer's instructions were followed, and reaction conditions were optimized for each primer pair. The primers used are summarized in the table 1. Following the PCR, amplified PCR products were analyzed by electrophoresis on 1% agarose gel to ensure correct amplification. PCR results were analyzed using CFX Manager Software.

4.8 Immunocytochemistry

For immunocytochemistry, 200 000 cells were seeded on a 6-well plate on the top of cover glass and transfected with *UBAP2* or control constructs as described above. Three days after the transfection cover glasses were removed and the cells were fixed with 4% paraformaldehyde. Cells were permeabilized using 0,5% NP-40/PBS solution. The fixed cells were probed with rabbit-anti-UBAP2 primary antibody and fluorescent-

labeled Alexa Fluor 594 goat-anti-rabbit secondary antibody. The cover glasses were mounted on object slides using Vectashield mounting media (Vector Labs) containing DAPI. Slides were imaged using Zeiss Axio Imager M2 fluorescence microscope.

CLONING PRIMERS	
Gene / exon	Primer sequence 5'-3'
	Forward
UBAP2 / exon 1	GAGAGCAGCAGCGATTTTCT
	Reverse
UBAP2 / exon 29	TACCCAAACAGCCTTGAACC
SEQUENCING PRIMERS	
Gene / exon	Primer sequence 5'-3'
	Forward
M13 Forward (-20)	GTAAAACGACGGCCAG
UBAP2 / exon 1	GAGAGCAGCAGCGATTTTCT
UBAP2 / exon 5	ATTCAGAAAACAAAGAGAAT
UBAP2 / exon 6	TGCAATCAAGTGGACAAACC
UBAP2 / exon 9-10	GTTCTTATGGACTCAAAGGG
UBAP2/ exon 12	CAACAATCAGATGGCACCAG
UBAP2 / exon 16	TGTCTTCCTCTTATGACCAG
UBAP2 / exon 20	CGGCAGCGACCTCCGTCTCA
UBAP2 / exon 24	ACTCTGCATCCCCTGCACCC
UBAP2 / exon 27	TATCAGTGTCTTCAAGCACC
UBAP2 / exon 28	TTTGACAAGCAGGGATTTCA
	Reverse
M13 Reverse	CAGGAAACAGCTATGAC
UBAP2 / exon 13	GTGGTGCAAGCTCTCCAAAT
UBAP2 / exon 16	TGGTTCCTGGAGCTGACTCT
UBAP2 / exon 28	GCTGGCAAGATGTGTAGGAA
UBAP2 / exon 29	TACCCAAACAGCCTTGAACC
RT-QPCR PRIMERS	
Gene / exon	Primer sequence 5'-3'
	Forward
TBP3	GGGGAGCTGTGATGTGAAGT
UBAP2 / exon 1	GAGAGCAGCAGCGATTTTCT
UBAP2 / exon 16	TGTCTTCCTCTTATGACCAG
UBAP2 / exon 17	TGGTCGAAGTCAGCAGACAC
	Reverse
TBP3	GAGCCATTACGTCGTCTTCC
UBAP2/ exon 3	ACGCATCTGTTCAGCTGTTG
UBAP2 / exon 16	TGGTTCCTGGAGCTGACTCT
UBAP2 / exon 18	GCTGAGAGAGAGGGCTGCTA

Table 1. Primer sequences. The left-hand panels shows primer name and its binding position, and the right-hand panel shows primer sequence.

5. RESULTS

5.1 Cloning of Full Length *UBAP2* and different *UBAP2* splice variants

In this study, the full-length cDNA of human *UBAP2* was successfully cloned. In addition, the cloning yielded multiple different *UBAP2* splice variants. The structures of fully sequenced variants are presented in the Figure 2. One of these splice variants, E1-6/9-29, contains all but two exons and it is probably translated to a slightly truncated UBAP2 protein. Similarly, the translation of the E29'/'14-29 variant probably results in N-terminally truncated UBAP2 protein. The rest of the variants produce C-terminally truncated proteins caused by premature stop-codons or alternative splicing. In some variants the splice site or sites differ from the typical 3' and 5' splice sites. Besides of the variants presented here, cloning yielded a dozen of different variants that we did not sequence completely.

5.2 Verification of UBAP2 overexpression by RT-qPCR

We subcloned the full length *UBAP2* and three truncated variants, E1-6/9-29, E1-4'/'29 and E29'/'14-29 to pCMV-Sport6 expression vector and overexpressed them in prostate cancer cells. The overexpression of *UBAP2* in transfected prostate cancer cells was verified by RT-qPCR. The results of the RT-qPCR experiments are presented in the Figure 3A. These results show that both PC-3 and LNCaP cells transfected with full-length *UBAP2* express *UBAP2* mRNA at high levels as compared with cells transfected with empty expression vector. The overexpression of *UBAP2* in cells transfected with truncated *UBAP2* variants was verified by using RT-qPCR with two different gene-specific primer pairs, each having priming sites in different parts of the mRNA molecule. Figure 3B-C shows that the cells transfected with variants E1-6/9-29 and E1-4'/'29 overexpress *UBAP2* exons 1-3 at low and high levels, respectively, when compared to the cells transfected with the E29'/'14-29 variant or empty expression vector. Correspondingly, the cells transfected with variants E1-6/9-29 and E29'/'14-29 overexpress *UBAP2* exon 16, unlike the cells transfected with the E1-4'/'29 variant or empty expression vector. Again, the expression level of *UBAP2* in the cells transfected

with variant E1-6/9-29 is lower than the expression level in the cells transfected with variant E29'/'14-29.

5.3 The effects of *UBAP2* expression on the growth of prostate cancer cells

The effects of *UBAP2* expression on cell growth were studied by using two different prostate cancer cell lines, PC-3 and LNCaP. Figure 4. shows the results of the cell growth experiments conducted by using PC-3 cells transfected with full length *UBAP2* (Figure 4A) or with truncated *UBAP2* variants (Figure 4B). In the case of the full length *UBAP2*, it seemed that the over expression of *UBAP2* slightly improved cell growth, but the difference was not statistically significant ($p > 0.05$). In the case of the truncated *UBAP2* variants, all studied variants seemed to significantly ($p < 0.05$) inhibit cell growth. Figure 5. shows the results of the cell growth experiment performed by using LNCaP cells transfected with full length *UBAP2* (Figure 5A) or with truncated *UBAP2* variants (Figure 5B). In case of the full length *UBAP2*, the effect is similar as with PC-3 cells and *UBAP2* seems to slightly improve cell growth when compared to the control cells. However, the difference in the growth rates of the cells was not statistically significant ($p < 0.05$). In the case of the truncated *UBAP2* variants, all variants seemed to slightly inhibit cell growth, but the difference was not statistically significant ($p < 0.05$).

5.4 Verification of UBAP2 overexpression at the protein level, and its localization by fluorescence immunocytochemistry

Fluorescence immunocytochemistry was applied to analyze UBAP2 protein expression and localization in prostate cancer cells transfected with full-length *UBAP2*. We observed that around 20% of the cell population overexpressed UBAP2 at the protein level. With LNCaP cells this fraction was slightly higher as compared with PC-3 cells. As expected, cells transfected with an empty expression vector did not overexpress UBAP2. We also observed that the localization of UBAP2 was strongly cytoplasmic. Representative fluorescence microscope images of PC-3 and LNCaP cells transfected with *UBAP2* are shown in the Figure 6.

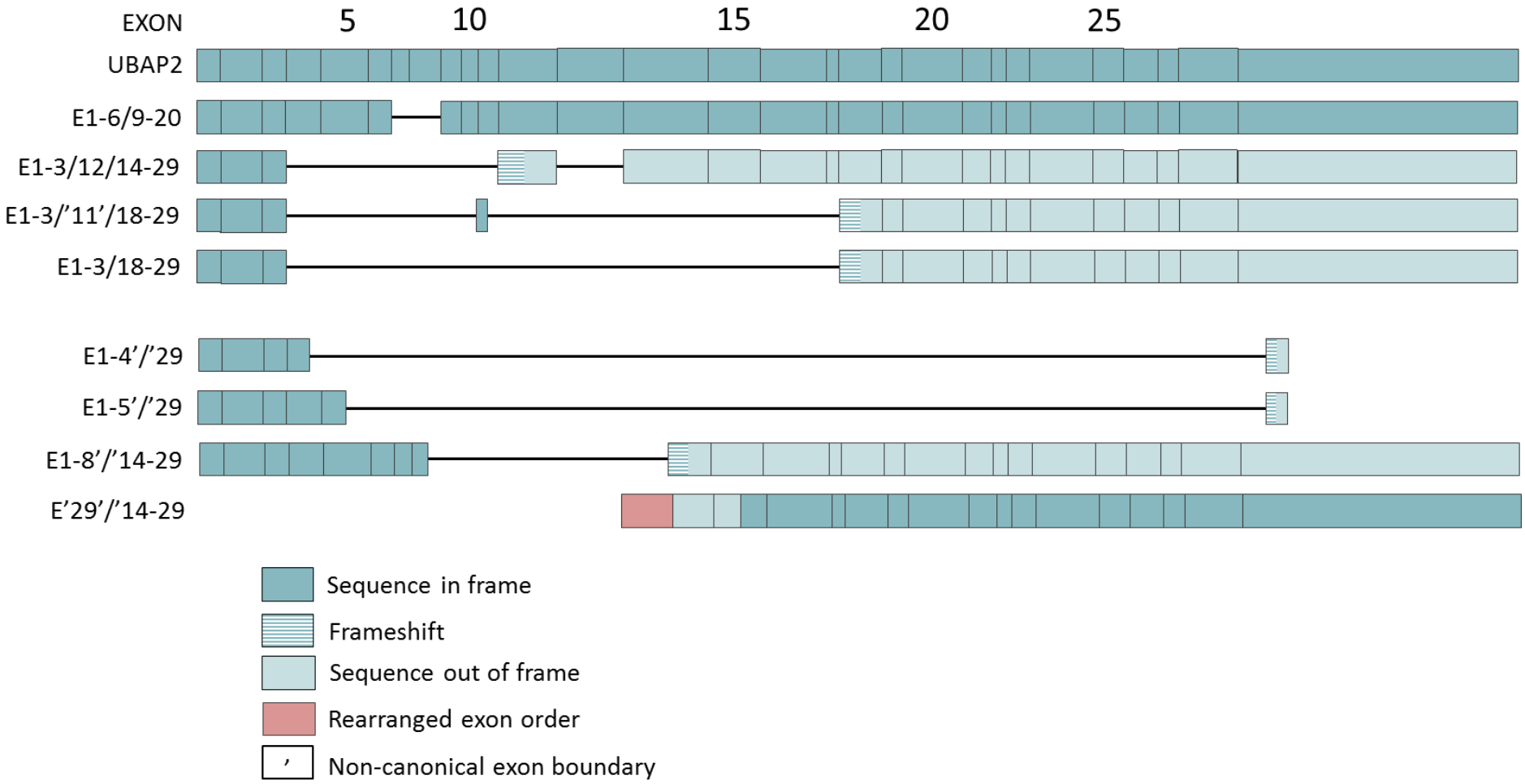

Figure 2. The exon structures of the cloned *UBAP2* variants. The uppermost bar represents full-length *UBAP2* cDNA and the lower bars represent truncated *UBAP2* variants. A darker shade of blue represents sequences that are in frame, a lighter shade of blue represents sequences out of frame. Stripes represent frameshifts. Red color represents rearranged exon order. Apostrophe (') represents non-canonical exon boundaries.

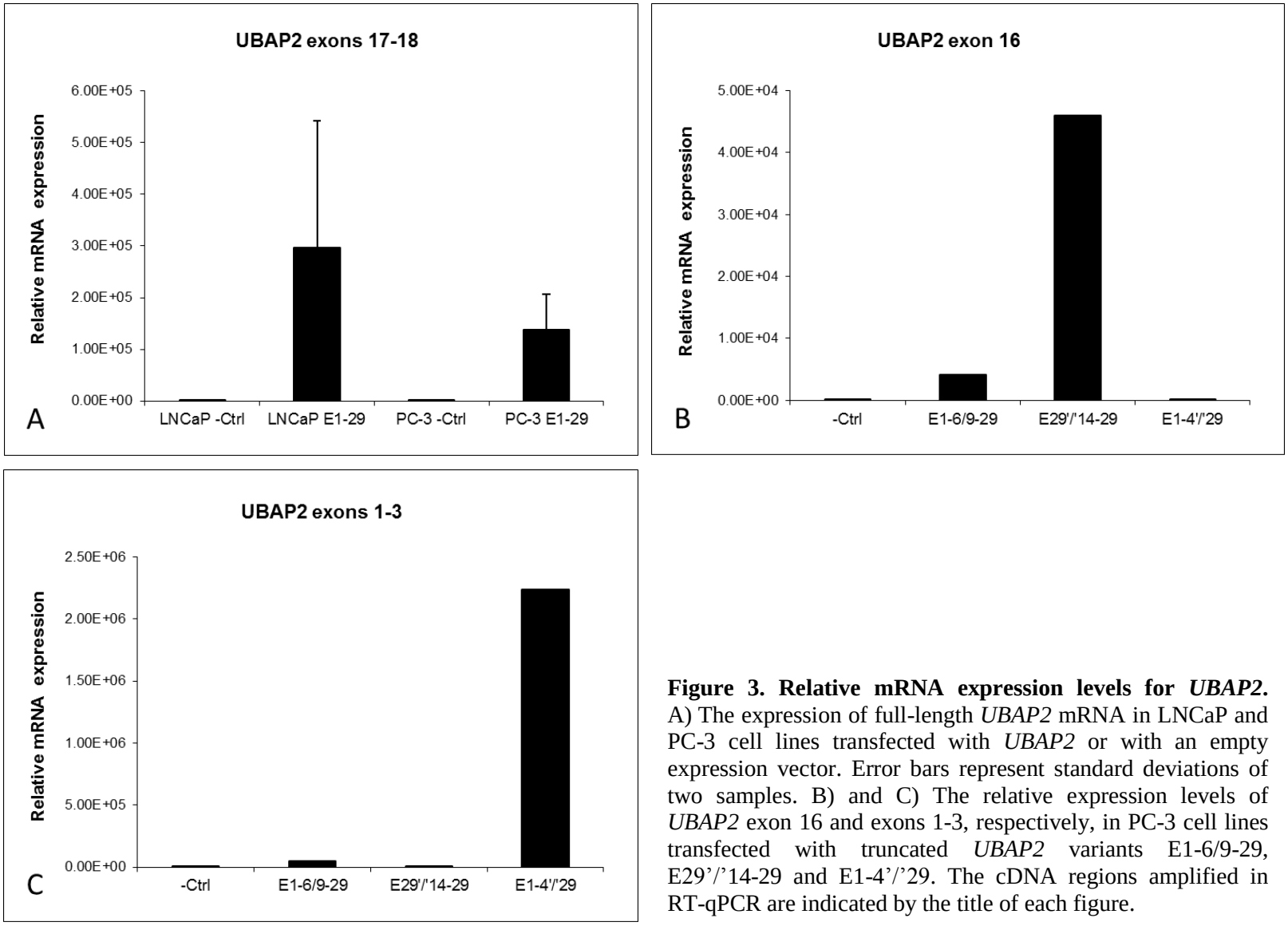

Figure 3. Relative mRNA expression levels for *UBAP2*. A) The expression of full-length *UBAP2* mRNA in LNCaP and PC-3 cell lines transfected with *UBAP2* or with an empty expression vector. Error bars represent standard deviations of two samples. B) and C) The relative expression levels of *UBAP2* exon 16 and exons 1-3, respectively, in PC-3 cell lines transfected with truncated *UBAP2* variants E1-6/9-29, E29'/'14-29 and E1-4'/'29. The cDNA regions amplified in RT-qPCR are indicated by the title of each figure.

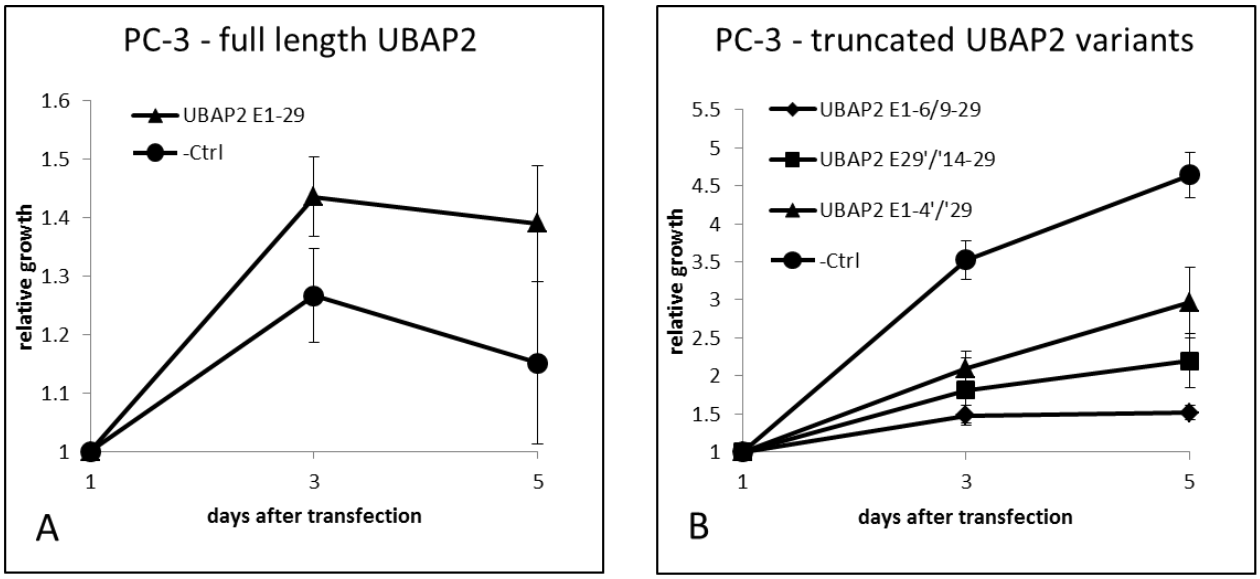

Figure 4. The relative growth of PC-3 cells transfected with *UBAP2* quantified by Alamar Blue assay. A) 20 000 PC-3 cells were seeded to 24-plate wells and transiently transfected to overexpress full length *UBAP2* or with empty expression vector. Cell growth was analyzed on the first, third and fifth day after transfection. B) 15 000 PC-3 cells were seeded to 24-plate wells and transiently transfected to overexpress three different *UBAP2* splice variants or with empty expression vector. Cell growth was analyzed on the first, third and fifth day after transfection.

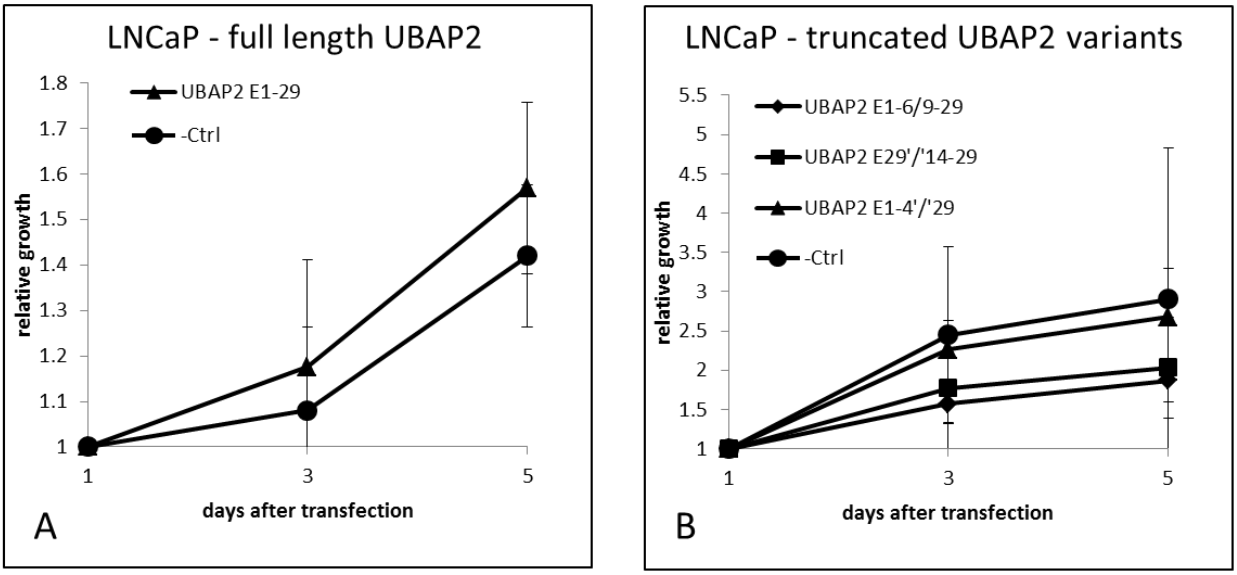

Figure 5. The relative growth of LNCaP cells transfected to express *UBAP2* quantified by digital imaging. A) 30 000 LNCaP cells were seeded to 24-plate well and transiently transfected to overexpress full length *UBAP2* or with empty expression vector. Cell growth was analyzed on the first, third and fifth day after transfection. B) 30 000 LNCaP cells were seeded to 24-plate well and transiently transfected to overexpress three different *UBAP2* splice variants or with empty expression vector. Cell growth was analyzed on the first, third and fifth day after transfection.

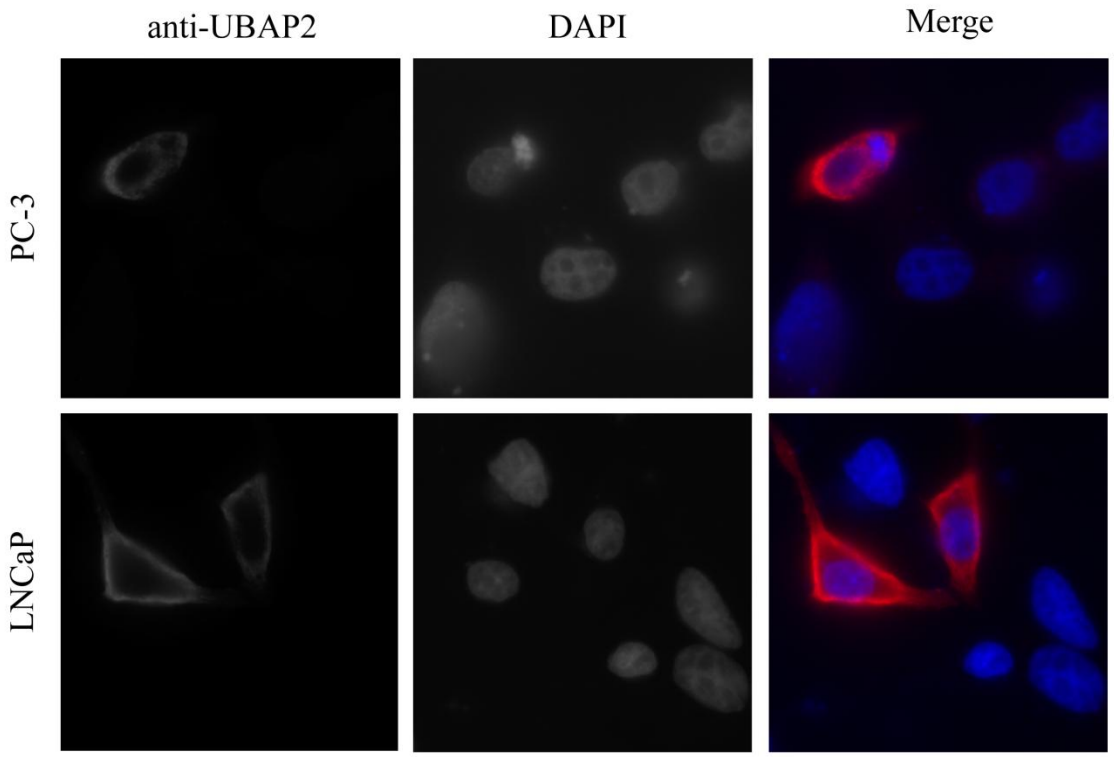

Figure 6. Over expression of UBAP2 in PC-3 and LNCaP cells transfected with full-length *UBAP2*. The upper row represents PC-3 and the bottom row represents LNCaP cells. The left-hand-side panel shows anti-UBAP2 immunostaining, the center panel nuclear DAPI staining and the right-hand-side panel shows merged images.

6. DISCUSSION

In this study, we cloned *UBAP2* gene and overexpressed it in prostate cancer cells. Previous studies carried out by our group have pointed out that chromosomal region 9p13.3 is recurrently amplified in prostate cancer. This amplification was also found to be associated with higher PSA-levels and poor progression-free survival in prostatectomy treated patients (Leinonen 2007). Chromosomal region 9p13.3 harbors multiple genes but none of them has known tumorigenic functions. For some of these genes, the level of the mRNA expression correlated with the copy number, thus making them candidate genes of that amplification (Leinonen 2007, Taylor et al. 2010). In the present study, we focused on one of these candidate genes locating in the 9p13.3 region, *UBAP2,* which encodes ubiquitin associated protein 2 (UBAP2). Function of this protein is unknown. UBAP2 contains one UBA-domain, which is known to bind mono- or polyubiquitin molecules. This domain is found from many proteins involving in ubiquitin-dependent signaling pathways.

According to previous studies conducted with clinical samples, the expression of *UBAP2* mRNA is significantly increased in prostate cancer compared to benign prostatic hyperplasia. In addition, down-regulation of *UBAP2* by siRNA significantly decreases the proliferation rate of prostate and breast cancer cells (unpublished data of this group). RNA sequencing results also show that *UBAP2* levels increase in castration resistant prostate cancer compared to prostate cancer (unpublished results). These previous results indicate that *UBAP2* is likely to be a notable factor in the regulation of proliferation of prostate cancer cells and supported the choice of *UBAP2* as the subject of this study.

In a previous study by Al-Balool et al., it was shown that *UBAP2* mRNA is a common target for post-transcriptional exon shuffling (PTES) events (Al-Balool et al. 2011). PTES events, also known as exon scrambling, are rearrangements of mRNA molecules originating from a single gene. In PTES rearrangements the exon-intron boundaries remain intact but the order of exons in the transcript differs from the genomic order of exons. The frequency and functional significance of PTES transcripts is in many cases still largely unknown. RNA sequencing studies from clinical prostate samples, in turn,

indicate that some *UBAP2* exons are expressed at different level in castration resistant prostate cancer as compared to benign prostatic hyperplasia (unpublished data of this group). For example, the exons from 4 to 7 are overexpressed in castration resistant prostate cancer. This might indicate that the splice variants containing these exons are more frequent in castration resistant prostate cancer than in benign prostatic hyperplasia.

6.1 Cloning of full-length *UBAP2* and splice variants

In this study, we cloned for the first time a cDNA coding for the human *UBAP2* gene. The cloning procedure of this gene was complicated by the huge number of *UBAP2* splice variants. When reverse transcribed RNA was amplified with *UBAP2* specific PCR primers, every reaction yielded several products, differing by the size and by the exon structure. These included transcripts processed by exon skipping, alternatively spliced exon 5' and 3' splice sites and intron retention. In fact, cloning of the full-length *UBAP2* cDNA proved to be quite challenging, as both PCR and TOPO-cloning seem to favor the truncated variants. It is possible that reason for this bias in the cloning reaction was technical, as shorter DNA strands are more easily amplified in PCR and somewhat easier to transform to cells. Nevertheless, it is also possible that RNA, which was our starting material for cloning, contain a greater amount of truncated *UBAP2* variants than full-length *UBAP2* mRNA.

In addition to the variants lacking exons in the middle of the structure, we cloned one transcript, E29'/'14-29, which was truncated from the 3' end. In this variant, there was a short, incorrectly positioned stretch of sequence from the exon 29 containing the binding site for the reverse primer used in the amplification of reverse transcribed *UBAP2* mRNA. This stretch of sequence is joined to the 3' side of the exon 14 in inverted orientation and complementary to the sense strand. It is not likely that this is due to nonspecific primer binding, as there is a short sequence from the exon 29 outside the primer binding site. Instead, it may have arisen from intragenic trans-splicing. In this alternative mode of splicing, two distinct pre-mRNA molecules encoding the same protein are joined together to form a single processed mRNA (Horiuchi, Aigaki 2006). This theory is supported also by the recent report describing that *UBAP2* is a common target for post-transcriptional exon shuffling (PTES) events. However, in this case, both 3' and 5' splice sites were non-canonical, which is atypical for PTES and trans-splicing

events. Moreover, sequence from the exon 29 was joined with 3' end of exon the 14 is in antisense orientation. This raises doubts about the possibility of experimental artifacts. A closer look at the sequence of *UBAP2* mRNA revealed, that there was a significant sequence similarity between the splice boundaries of both splice donor and acceptor (data not shown). This suggests that observed transcripts may have resulted from an experimental artifact caused by reverse transcriptase template switching during cDNA preparation (Houseley, Tollervey 2010).

Template switching is a process, in which reverse transcriptase jumps to another location within the template without terminating DNA synthesis (Houseley, Tollervey 2010, Cocquet et al. 2006). Template switching can also occur *in vitro* during cDNA synthesis. Reverse transcriptase template switching has been implicated in the untypical intramolecular splicing events that are sometimes wrongly interpreted as intramolecular trans-splicing processes. The key indicators of this process are non-canonical splice sites and high sequence identity between the donor and acceptor sites. Template switching can also result in trans-splicing between sense and antisense transcripts, as reverse transcriptase can jump from mRNA to the cDNA being produced by another enzyme from the same template (Houseley, Tollervey 2010). All of the considerations presented above support the hypothesis that the E29'/'14-29 transcript is a result of reverse transcriptase template switching event that has occurred *in vitro* during the cDNA synthesis.

This observation lead us to question if some other *UBAP2* variants are false transcripts as well. A more detailed look at the sequences pointed out that most of the variants having non-canonical splice sites also had high sequence identity between the donor and the acceptor sites. On the contrary, the splice sites of the variants with canonical exon boundaries had no notable sequence identity. This strongly indicates that template switching events have taken place in the reverse transcription of *UBAP2* splice variants with non-canonical exon boundaries. On the other hand, the splice variants with canonical exon boundaries are likely to have resulted from regular cis-splicing in the cells.

Reverse transcriptase template switching is a relatively rare event that causes artifacts mainly in high throughput experiments that produce a huge volume of transcriptome

data (Houseley, Tollervey 2010). However, some mRNAs, like *FOXL2*, are reported to be more prone to the template switching than others (Cocquet et al. 2006). The reason may be the mRNA secondary structure, which brings two separate homological regions close to each other. Also the high number of homological regions may increase the likelihood of the template switching. According to our results, *UBAP2* seems to be prone to template switching. In the future studies, it would be reasonable to try to reduce template switching, as it seems to be a recurrent artifact in the cDNA synthesis of *UBAP2*. The use of different reverse transcriptases might help to avoid template switching, as Super Script and AMV reverse transcriptases have been shown to have the different template switching propensities (Houseley, Tollervey 2010). An increase in the reaction temperature has also been reported to inhibit template switching (Cocquet et al. 2006). Two *UBAP2* false variants, E1-4'/'29 and E29'/'14-29, encode N- and C-terminally truncated UBAP2 protein, respectively.

The presence of different mRNA variants can be studied by northern blot analysis. In this method, total RNA extracted from cells or tissue sample is separated by agarose gel electrophoresis, blotted to a membrane and detected with a DNA probe labeled with radioactive phosphorus. Since the RNA molecules are separated according to their size within the agarose gel, one should be able to detect all variants with different sizes, as long as they contain the region recognized by the probe. If RNA ladder is used, also the size of the detected RNA molecules can be analyzed. Despite of its efficiency in detecting mRNA molecules, northern blot analysis is a complex method and the quality of starting material plays a major role in the success of analysis. In this study, we tried to detect different *UBAP2* splice variants by using northern blotting, but the analysis of the results was prevented by the low intensity of the bands and by the high background signal (data not shown). The reason for these results could be technical, e.g. poor quality of RNA or poor probe design. It is also possible that the levels of *UBAP2* variants in the samples were very low and fell below the detection limit.

6.2 Overexpressing *UBAP2* in prostate cancer cells

Our aim was to study whether the overexpression of *UBAP2* has effects on the growth and proliferation of prostate cancer cells. To do this, we transfected cells with an expression plasmid carrying the *UBAP2* gene. We also ensured the efficiency of the

transfection method before carrying out other experiments. We verified the overexpression of *UBAP2* at the mRNA-level by RT-qPCR. In this method, RNAs extracted from cells are converted to complementary DNA, and the amount of DNA is quantified during PCR-amplification by utilizing fluorescent dyes binding to double stranded DNA. By using RT-qPCR, we were able to measure the amount of *UBAP2* mRNA in cells transfected with *UBAP2* gene and compare it to that of the cells transfected with control vector.

UBAP2 was overexpressed under cytomegalovirus (CMV) promoter, which typically induces a very strong expression. Therefore, artificial overexpression might be several-fold higher than naturally occurring levels of expression. According to RT-qPCR results, cells transfected with either full-length or truncated *UBAP2* variants overexpress it at mRNA-level, and the difference to the cells transfected with empty expression vector was significant. However, there were significant differences in the expression levels between different truncated variants. Variant E1-6/9-29 was overexpressed at a much lower level when compared to variants E1-4'/'29 and E29'/'14-29. The reasons for these differences in the expression levels are still largely unknown. Variant E1-6/9-29 is significantly longer (4130 bp) than the other variants (E1-4'/'29 is 406 bp and E29'/'14-29 is 2901 bp). We transfected cells with equal mass amount of DNA, which result in higher molar amount of shorter DNA strand and in higher plasmid copy number in the cells. Anyhow, the difference caused by this is quite minor (about 2 fold) and should not cause large variations in the expression level of different splice variants. It is also possible that the transfection efficiency is higher for shorter DNA strands. In addition to variations in the rates of mRNA synthesis, there could be differences in the degradation rates of different mRNA molecules. Hence, it is possible that E1-6/9-29 *UBAP2* variant is degraded at a much higher rate than shorter truncated variants. These kind of differences in the turnover rates of different mRNA variants might be caused by the presence of different stabilizing and destabilizing elements within different mRNA molecules.

The cell lines we used for the overexpression and proliferation studies were LNCaP and PC-3. Both of these cell lines are widely used human prostate adenocarcinoma cell lines. According to previous studies carried out by our group, LNCaP cells are known to

harbor low-level amplification of 9p13.3, whereas the copy-number status of PC-3 cells is normal. This means that also the LNCaP cells not transfected with *UBAP2* might express it at moderate level. According to our RT-qPCR results, *UBAP2* expression level of both LNCaP and PC-3 cells transfected with empty expression vector was negligible as compared to the cells transfected with *UBAP2*.

6.3 Biological effects of UBAP2

Even though the overexpression of full-length *UBAP2* seems to have a small improving effect on the growth rate of prostate cancer cells, we could not point out statistically significant difference. It is possible, simply, that function of UBAP2 in the cell has no direct effect on mechanisms regulating cell proliferation and thus overexpression of *UBAP2* in the cells has no apparent effects on the short time scale. Alternatively, UBAP2 might effect on a pathway that saturates already at low UBAP2 concentration. In that case, extra protein has no impact on this pathway, if the endogenous UBAP2 has already saturated it. It is also possible that UBAP2 has an effect on the growth only in the presence of some other, thus far unknown factor. However, it is possible that our study was unable to find the possible effect of UBAP2. The main reason for this could be the overexpression method utilized in this study. Full-length *UBAP2* and various truncated variants were overexpressed by using transient transfection. In this method, transfected DNA is not integrated into the genome of the host cell, but remains as a epichromosomal plasmid which is eventually lost from the cell as the cell proliferates. Moreover, there was no selection to distinguish non-transfected cells from those that have received plasmid. However, transient transfection method is fast and simple to perform, and that is why it was selected in the current study.

According to the results of the fluorescence immunocytochemistry experiments conducted in this study, less than half of the cell population transfected with full-length *UBAP2* actually overexpressed it. It is also commonly known that this is weakness of transient transfection. If only a small portion of the population has improved proliferation ability, studying the whole population may not reveal it. Another weakness of transient transfection method is that transfection reagents are sometimes harmful for cells. The transfection process itself affects the viability of the cells, so it is possible they do not behave completely normally as without transfection. The bias caused by this

effect can be at least partially neutralized by transfecting control cells with empty expression vector, as we did. For the reasons presented above, it could be reasonable to create stable clones overexpressing *UBAP2* in the future studies.

As opposed to full-length *UBAP2*, truncated *UBAP2* variants inhibited the proliferation of PC-3 cells when overexpressed in them, and this effect was statistically significant. This seems unexpected, as we hypothesized that truncated UBAP2 variants might have partially similar features as full-length protein. The only known functional domain of UBAP2, the UBA-domain, is encoded by the exons 3 and 4, and the two of three studied truncated variants contain this area. For this reason, it seems unlikely that all of the three different UBAP2 variants analyzed in this study would have inhibitory features lacking in full-length UBAP2. However, it is possible that these truncated variants are misfolded in the cell and overexpression of misfolded proteins itself is a burden for the cell. There is the accurate protein degradation mechanism in the cell and usually it effectively degrades all the misfolded and damaged proteins preventing them to disturb the functions of the cell (Goldberg 2003). If the production of misfolded proteins exceed the cell's degradative capacity, proteins commonly aggregate. These protein aggregates are cytotoxic and inhibitory effect of truncated UBAP2 variants could arise from this phenomenon.

As the biological function of UBAP2 is still largely unknown, we can only speculate if the truncated UBAP2 proteins have any function in the cells. The results of this study do not assess whether the truncated *UBAP2* transcripts are translated and expressed at the protein level in the cells. If they are translated, it is possible that they fold incorrectly and are very short-lived and unstable. In spite of all, if these variants are translated and folded, they possibly have same UBA-domain mediated function as full-length UBAP2 protein, at least partly. However, it is unknown if UBAP2 function is dependent on UBA-domain.

Ubiquitin associated domains (also known as UBA-domains) are functional protein domains that are able to bind ubiquitin molecules. Ubiquitin is a small regulatory protein, which is in most cases attached to proteins thus labeling them for degradation (Su, Lau 2009). This process called ubiquitination regulates a wide range of cellular functions in eukaryotes. Attachment of a chain of multiple ubiquitins, polyubiquitin,

usually labels proteins for degradation, while the attachment of a single ubiquitin, monoubiquitin, is in some cases associated with non-proteolytic functions (Hicke 2001). The UBA-domain family was first described by Hofmann and Bucher over 15 years ago (Hofmann, Bucher 1996). The length of the primary structure of these domains is about 45 amino acids. UBA-domains are quite common in proteins. In some cases, the biological functions of the UBA-domain containing proteins are well-known, while the functions of others are only poorly understood. Generally, proteins containing UBA-domain are frequently involved in ubiquitin-dependent signaling pathways. UBA-domains can be classified according their binding properties; some of them interact strongly with polyubiquitin chains or monoubiquitin molecules, while the others interact only with polyubiqitin chains linked to specific molecules. However, there are no studies that the UBAP2 protein would have any ubiquitin-dependent functions, but due to its UBA-domain this possibility cannot be ruled out.

The results of the fluorescence immunocytochemistry experiment pointed out that only a small portion of transfected cells overexpressed UBAP2, but they also indicate that the full-length UBAP2 was highly overexpressed also at protein level. The expressed UBAP2 proteins localized predominantly to the cytoplasm. Unfortunately, the lack of commercial UBAP2 specific antibodies suitable for western blot prevented us from measure UBAP2 protein level expression by this method. The only available anti-UBAP2-antibody at the time of our study was guaranteed by manufacturer only for immunohistochemistry. We tested it also to western blot, but it seemed not to work, as results were unreadable and messy. This might result from the fact that proteins are denatured for western blotting, unlike in immunocytochemistry. Some antibodies recognize only correctly folded target protein, and anti-UBAP2-antibody used in this study may belong to this group. Nowadays, at least one new anti-UBAP2-antibody has been launched on the market, and it is guaranteed to be applicable to western blot also. In the future, it would be reasonable to use it for the detection of UBAP2 in western blot.

7. CONCLUSION

The aim of this study was to clone *UBAP2* and to explore its role in prostate cancer. *UBAP2* is a potential target gene of the 9p13.3 chromosomal gain previously identified by our group. In this study, we cloned the full-length *UBAP2* gene and some of its truncated variants. We also identified multiple novel splice variant of *UBAP2*. In addition, we noticed that *UBAP2* mRNA seems to be prone to template switching artifacts during cDNA synthesis. In the future, there will be a need to determine the function of this poorly known gene, which would also open possibilities to understand the role of variable splicing.

The second aim of this study was to analyze the effects *UBAP2* overexpression has on the proliferation of prostate cancer cells. Our results suggest that full-length *UBAP2* might exhibit a small improving effect on the proliferation of prostate cancer cells. However, we cannot conclude from these findings whether *UBAP2* is the target gene of the 9p13.3 amplification. Further investigations have to be carried out to clarify the role of the increased expression of *UBAP2* in prostate cancer. Nevertheless, the results of this study provide a solid foundation for further studies focusing on the role of *UBAP2* in prostate cancer.

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