Faculty of Graduate Studies
An-Najah National University

(ETS) Family Factors: Role and Cooperative Interactions in Prostate Cancer

By
Jamila Sameer Zaki Daraghmeh

Supervisors
Dr. Nael Abu Hasan
Prof. Dr. Nicolas Wernert

This Thesis is Submitted in Partial Fulfillment of the Requirements for the Degree of Master in Life Sciences (Biology), Faculty of Graduate Studies, An-Najah National University, Nablus, Palestine.

2011

(ETS) Family Factors: Role and Cooperative Interactions in Prostate Cancer

Prepared by
Jamila Sameer Zaki Daraghmeh

This Thesis was defended successfully on 29/11/ 2011 and approved by:

Defense Committee Members		**Signature**
- Dr. Nael S. Abu-Hasan	**(Supervisor)**	.....
- Prof. Bashar M. Saad	**(External Examiner)**	.....
- Dr. Kamel Adwan	**(Internal Examiner)**	.....

Dedication

To My Family with Respect and Love

Acknowledgments

First of all, I would like to express my deepest sense of gratitude to my supervisor Dr. Nael Abu-Hasan for his patient guidance, encouragement and most importantly for reading and approving the thesis.

I would like to express my gratitude to Prof. Nicolas Wernert and his team for giving me the opportunity to conduct the scientific experiments in his lab in the Institute of Pathology - University Hospital Bonn University and for his support; however the thesis was read and approved only by Dr. Abu-Hasan.

My sincere thanks to faculty members at Science Department of Arab American University of Jenine for granting me the opportunity to peruse my master's degree programme.

Thank for faculty members of Graduate Studies at An-Najah national University for their efforts and support during my Master programme.

Finally, I take this opportunity to express my profound gratitude to my beloved parents, husband, brothers and sisters and son for their moral support and patience during my studies.

الإقرار

أنا الموقع أدناه مقدم الرسالة التي تحمل العنوان:

(ETS) Family Factors: Role and Cooperative Interactions in Prostate Cancer

الدور التكاملي لعناصر النسخ في سرطان غدة البروستات

اقر بأن ما اشتملت عليه هذه الرسالة إنما هو نتاج جهدي الخاص، باستثناء ما تمت الإشارة إليه حيثما ورد، وان هذه الرسالة ككل، أو أي جزء منها لم يقدم من قبل لنيل أية درجة علمية أو بحث علمي أو بحثي لدى أية مؤسسة تعليمية أو بحثية أخرى.

Declaration

The work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's name: …….....…… اسم الطالب:

Signature: ……..……..... التوقيع:

Date: …… التاريخ:

List of Contents

List of Tables

List of Figures

List of Abbreviations

DBDs	DNA-Binding Domains
HAT	Histone Acetyltransferase
HDAC	Histone deacetylase
GTFs	General Transcription Factors
HSF	Heat Shock Factor
ETS	E-Twenty Six
PC3	Prostate Cancer cell line
GFP	Green Fluorescence protein
HIF	Hypoxia Inducible Factor
TAL effectors	Transcription-Activator Like effectors
PBS	Phosphate Buffer Saline
siRNA	Small interference RNA

(ETS) Family Factors: Role and Cooperative Interactions in Prostate Cancer

Prepared by
Jamila Sameer Zaki Daraghmeh
Supervisors
Dr. Nael Abu Hasan
Prof. Dr. Nicolas Wernert

Abstract

In humans, the ETS family of transcription factors consists of 27 members that contain a highly conserved DNA binding domain. Members of this family are involved in many biological processes in both normal and neoplastic cells and the expression profile of the ETS family is well studied. Reported differences in the expression of ETS family members using different cell lines seems to reflect specific cellular characteristics of particular prostate cancer cell lines. Differential expression of ETS family members in prostate cancer tissues and androgen-sensitive and insensitive prostate cancer cell lines showed that ETS-1 regulates five family members in PC3 cells ELf-1, Elf-2, Elk-1, Etv-5 and Spi-1using Ets-1 blocked. In the current study, we tried to knockdown the ETS family members ETS-1 and ETS-2 in PC3 and DU-145 prostate cancer cell lines, respectively using shRNA plasmids in an attempt to achieve a stable knockdown.

Transfection of both cell lines was successful as indicated by green Fluorescence. Data analysis of the qRT-PCR results for ETS -1 indicate that knock- down of this gene was not possible. It seems reasonable to assume that the knock- down of this gene was not possible due to their involvement in the RNAi pathway, feedback mechanisms, or other

experimental components may need to be optimization (e.g. shRNA plasmid concentrations).

Knock- down of ETS-2 gene was successful; however, the gene was only transiently expressed. The next priority is creating a stable cell line in order to study biological properties of transfected cells such as migration, invasion, and proliferation of the cancer cells.

Chapter One

Introduction

1.1 General background

Transcription defined as the process of synthesis of RNA from a DNA template. The process whether prokaryotic or eukaryotic, has three main events: initiation stage which is considered as the most important step in gene expression and involve RNA polymerase binding to the promoter region; elongation stage represent the covalent addition of nucleotides to the 3' end of the growing polynucleotide chain and leads to single stranded molecule known as pre-mRNA and termination which is achieved by the recognition of the transcription termination sequence and the release of RNA polymerase.

Although transcription is performed by RNA polymerase, the enzyme needs other proteins to produce the transcript. These factors are either associated directly with RNA polymerase or involved in building the actual transcription apparatus. The general term for these associated proteins is transcription factor.

Transcription factors are proteins other than RNA polymerase that is required for transcription and function in any one of the following ways:

- bind to RNA Polymerase
- bind another transcription factor
- bind to cis-acting DNA sequences

RNA polymerase and the group of protein that directly interact with it are called the basal transcription apparatus. This is the apparatus that is directly responsible for transcription [1].

Basal transcription apparatus includes RNA polymerase and general factors; both needed for initiation of transcription. Other factors, those that interact directly or through a co-activator with the proteins of the basal transcription apparatus, are also important for transcription. These generally have a positive effect on transcription, but occasionally they can repress gene expression through transcription. These factors are called upstream factors that seem to increase the efficiency of transcription initiation. Each promoter seems to have a set of unique factors. The functions of these factors include:

- influence the initiation of transcription by contacting members of the basal apparatus
- promotes assembly of the apparatus
- may bind co-activators that interact with the basal apparatus
- typically bind to TFIID, TFIIB or TFIIA
- TFIID provides various TAFs that can be interacted with; if TAFs are unique to a specific promoter, then the interaction can control promoter specific transcription
- most interactions are positive in nature and induce transcription
- repressors may prevent the building of the basal apparatus

These factors are turned in a temporal or spatial manner, or directly in response to the environment and provide the final link in controlling gene expression. They are termed inducible factors and act in the same

manner as an upstream factor but their synthesis is regulated in a temporal or spatial manner [2].

Given all of the discussion regarding the basal transcription apparatus, and upstream and inducible factors, it is important now to define the promoter, which reflects the interaction of the all of the important proteins and the DNA to which they bind. Thus, promoters refer to all the DNA sequences containing binding sites for RNA polymerase and the transcription factors necessary for normal transcription.

1.2 Transcriptional units - genes

The discovery of RNA splicing during 1970s and the findings that a single mRNA molecule hybridizes not to a single stretch of DNA but to as many as four or more discontinuous DNA segments along the genome [1, 2] clearly contradict the proposed structure of a functional gene consisting of many sites arranged strictly in a linear order as suggested by crick [3]. These discoverers showed that genes can be split into several segments along the genome.

Studies on most eukaryotes indicate that discontinuous gene structure and splicing during RNA processing are the norm, not the exception. Some vertebrate genes contain as many as 50 exons and exons often make up only a small portion of the transcribed region of a gene. For example, in one early splicing study that involved examination of the intron-exon pattern of a chicken ova albumin gene, measured eight exons ranging in length from 20 to 181 base pairs and seven introns ranging in length from 264 to 1,150 base pairs [4].

The final protein products encoded by any given intron-exon sequence also vary in structure, depending on which exons are spliced back together during RNA processing in a process called "alternative splicing". Scientists have also since learned that eukaryotic cells have evolved another "alternative" mRNA processing pathway: the use of multiple 3' cleavage sites in a single exon. The end result is the same as with alternative splicing: different mRNA molecules are produced from a single protein-coding gene. Clearly, contrary to the conventional notion of a single gene encoding a single protein, a single continuous stretch of DNA can encode multiple mRNA molecules and, ultimately, multiple protein products.

Given the vast quantity of DNA that appears to have little protein-encoding power and the fact that so much of this DNA resides right in the middle of functional genes (as introns), some scientists prefer to think in terms of "transcription units" rather than "genes." A transcription unit is a linear sequence of DNA that extends from a transcription start site to a transcription stop site [1, 2].

1.3 Promoters and enhancers

The promoter, a DNA sequence that lies upstream of the RNA coding region, serves as an indicator of where and in which direction transcription proceed. The promoter is not actually transcribed; its role is purely regulatory. While promoters vary tremendously among eukaryotes, there are some common features. For example, most promoters lie immediately upstream of the transcription unit (transcription proceeds in an upstream to downstream direction), and most contain what is known as a

TATA box; this is a sequence that is recognized and bound by a so-called TATA binding protein. The TATA binding protein helps position the RNA polymerase machinery and initiates transcription. Some promoters work in concert with other types of regulatory sequences known as enhancers, which sometimes lay several kilo bases further upstream or downstream from the coding sequence itself, or even within introns. These two sequences are able to interact because of the way DNA molecules bend in space, enabling sections that would otherwise be very far from each other to interact (via DNA-binding proteins). Enhancer regions serve as binding sites for proteins known as activators. The proteins that bind to promoters to regulate transcription are called transcription factors. The RNA coding region, the main component of the transcription unit, contains the actual exons and introns. The terminator, a sequence of nucleotides at the end of the transcription unit, is transcribed along with the RNA coding region. The terminator serves as a speed bump of sorts; transcription stops only after this region has been transcribed [5].

Scientists have recently discovered that some mRNA molecules are coded by exons from multiple transcription units through a process known as trans-splicing. In fact, in 2005, a European group of researchers estimated that about 4% to 5% of tandem transcription units (i.e., distinct but adjacent transcription units) in humans are transcribed together to create single "chimeric" mRNA molecules. Scientists are not sure how this occurs. Some speculate that transcription overrides the first transcription terminator and doesn't stop until it reaches the second termination site; others suspect that both transcripts are formed independently and then spliced together to form the chimeric mRNA molecule [5].

1.4 Eukaryotic transcription

Compared to prokaryotes, eukaryotic transcription is more complex and every eukaryotic gene is unique requiring its own transcription machinery. Promoters for different genes are different; each contains a combination of sites to which specific protein factors bind. All of these factors help RNA polymerase to bind in the correct place and to initiate transcription.

There are three distinct RNA polymerases located mainly in eukaryotic cell nucleus which define three major classes of eukaryotic transcriptional units. RNA polymerase I transcribe rRNA except for 5SrRNA; RNA polymerase II transcribe hnRNA (pre mRNA) from nicked dsDNA template or from ssDNA template and RNA polymerase III transcribe small tRNA and 5SRNA.

Each of these RNA polymerases is a complex of many polypeptide subunits that cannot find or bind to a promoter by themselves. Each requires the binding of assembly factors and a positional factor to locate the promoter and to orient the polymerase correctly

1.4.1 Class I transcriptional units

Class I genes or transcriptional units are transcribed by RNA polymerase I and each unit consists of 3 rRNA genes: 18S, 5.8S, and 28S; and each unit is separated by a non transcribed spacer. RNA polymerase I is a complex of 13 polypeptide subunits. The core promoter region is located from -31 to +6 around the transcription start point. Another GC-rich sequence further upstream, called the upstream control element (UCE),

located from -187 to -107 is also required for efficient transcription. In general, sequences around the start-point of transcription tend to be AT-rich so that melting of the DNA duplex is easier.

Two additional transcription factors are known to assist RNA polymerase I known as UBF1 and SL1. UBF1 is a single polypeptide which binds to the upstream control element and to the core promoter. This factor recognizes the GC-rich sequences within these elements and considered as an assembly factor, while SL1 binds to UBF1 and consists of 4 proteins, one of which is TATA-box binding protein (TBP).

TBP is required for the assembly of a transcriptional complex in all 3 classes of eukaryotic transcription unit. SL1 is a positional factor - it targets RNA polymerase at the promoter so that it initiates transcription in the correct place. Once UBF1 and SL1 have formed a complex, RNA polymerase I bind to the core promoter to initiate transcription [4].

1.4.2 Class II transcriptional units

All genes that are transcribed and expressed via mRNA are transcribed by RNA polymerase II which is a complex of multi subunits that could reach 12 subunits as in yeasts.

Promoters used by RNA polymerase II have different structures depending upon the particular combination of transcription factors that are required to build a functional transcriptional complex at each promoter. Nevertheless, these different structures can be viewed as a combination of a relatively limited number of specific sequence elements. Some of the

common elements that have been described in class II eukaryotic promoters are the following:

- The TATA Box (TATAAAA) located about 25bp upstream of the start-point of transcription and found in many promoters

- The Initiator is a sequence that found in many promoters and defines the start point of transcription

- The GC box (GGGCGG) is a common element in eukaryotic class II promoters and may be present in multiple copies located between 40 and 100bp upstream of the start point of transcription.

- The CAAT box (CCAAT) is often found between 40 -100bp upstream of the start point of transcription.

- Enhancers may be required for full expression and not considered as a part of the promoter. They can be located upstream or downstream of the promoter and may be quite far away from it. The mechanism by which they work is not known. They may provide an entry point for RNA polymerase or they may bind other proteins that assist RNA polymerase to bind to the promoter region [4].

At least six general (or basal) transcription factors (TFIIA, B, D, E, F and H) have been characterized for class II transcriptional units. In the presence of these transcription factors, RNA polymerase II is able to initiate transcription at promoters correctly. However, even in the presence of transcription factors, the enzyme complex is unable to recognize and respond to regulatory signals as it seems to be affected by the presence of

promoter-proximal regulatory sequences to which transcription factors bind.

The basic process of assembly of a transcriptional complex is likely to include the following steps:

- TFIID recognizes and binds to the TATA box through TATA box Binding Protein (TBP) and ~10 TBP associated factors - TAFs. TBP is a small polypeptide of 180 amino acids with "saddle-shaped" structure that sits on DNA molecule and binds to it via contacts in the minor groove. The binding causes 80° bend in DNA.

- TFIIA binds and stabilizes TFIID binding. The RNA polymerase II holoenzyme assembles to form a pre initiation complex. The holoenzyme consists of the RNA polymerase II complex, the regulatory complexes and the following transcription factors:

- TFIIB is a single polypeptide. It can bind both upstream and downstream of the TATA box. It recruits TFIIF-RNA polymerase II to the complex or it may interact directly with the enzyme

- TFIIE is a complex of two subunits. It recruits TFIIH to the complex thereby, priming the initiation complex for promoter clearance and elongation

- TFIIF also has two subunits - RAP38 & RAP74. The latter has a helicase activity and may therefore be involved in melting the DNA at the promoter to expose the template strand

- TFIIH is a complex of 9 subunits. One of the subunits has a kinase activity that carries out the phosphorylation that is required for promoter clearance.

Data presented in figure 1.2 shows the order of assembly of transcription factors and the following order were suggested TFIID -> TFIIA -> TFIIB -> (TFIIF + RNAP II) -> TFIIE -> TFIIH [6]. There is also evidence that an additional transcription factor, TFIIS, participates in transcription elongation.

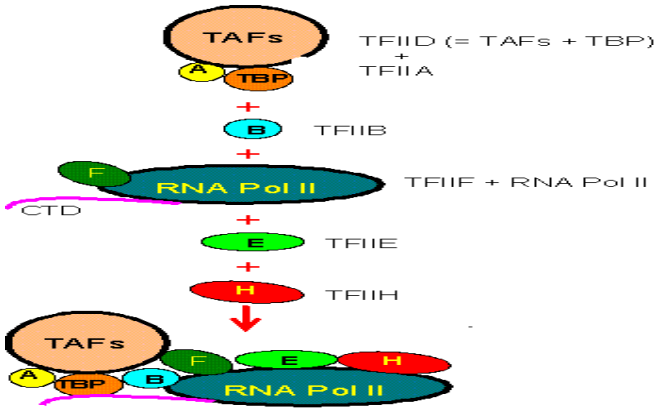

Figure 1.1 Proposed order of assembly of transcription factors in class II transcriptional units [6]

1.4.3 Class III transcriptional units

Class III genes are principally those for small RNA molecules. The best studied examples are the 5S rRNA genes. RNA polymerase III is the largest of the three RNA polymerases with 17 subunits and a molecular weight of over 700 KD.

Promoters for this unit are distinctive as some located within the gene they direct its transcription. The promoters for snRNA genes lie upstream of the start point of transcription.

Assembly of a functional complex requires the participation of a number of additional transcription factors. The following transcription factors have been characterized:

- TFIIIA - required only for the transcription of 5S rRNA genes and functions as an assembly factor for some class III promoters
- TFIIIB – consists of three subunits, one of which is TBP - TATA-box binding protein and considered as a positional factor
- TFIIIC - consists of 6 subunits and functions as an assembly factor. It appears to be required for all internal class III promoters.

The following represent the assembly of the transcriptional complex which proceeds in a step-wise manner:

TFIIIA binds to a site within the promoter region - TFIIIC binds to form a stable complex and covers the entire gene - TFIIB can now bind to its

binding site surrounding the start point of transcription and finally RNA polymerase III is able to bind and begin transcription [4].

1.4.4 RNA processing

The initial RNA molecules that are transcribed in the nucleus must often be processed after being synthesized and before leaving the nucleus to the ribosomes in the cytoplasm. The followings represent the three types of modifications that take place:

- Addition of about 200 adenine residues to the 3' end to form a poly A Tail
- Capping - the addition of 7-methylguanosine to the 5' end
- Splicing of introns and joining of exons

1.5 Transcription factors

Transcription factors are group of proteins that binds to specific DNA sequences and involved in the control of the flow of genetic information from DNA to mRNA in a process known as transcription [7, 8]. Many of the transcription factors act by recognizing cis-acting sites that are parts of promoters or enhancers. However, binding of transcription factor to DNA is not the only means of action as others may recognize another factor, or may recognize RNA Polymerases. Thus, transcription factors either work alone or with other proteins in a complex, by promoting *(e.g.* an activator), or blocking (e.g. a repressor) the recruitment of RNA polymerase to specific genes [9, 10, 11].

The main feature of transcription factors is that they contain one or more DNA-binding domains (DBDs), which bind to specific sequences of DNA adjacent to the genes that they regulate [12, 13]. They are essential for the regulation of gene expression and found in all living organisms. The number of transcription factors found within an organism increases with genome size, so larger genomes have more transcription factors per gene than smaller one [14]. Approximately 2600 proteins in the human genome that contain DNA-binding domains function as transcription factors [15].

About 10% of the genes in the genome code for transcription factors, furthermore genes are often flanked by several binding sites for distinct transcription factors, and efficient expression of each of these genes requires the cooperative action of several different transcription factors. Hence, the combinatorial use of a subset of the approximately 2000 human transcription factors easily accounts for the unique regulation of each gene in the human genome during development [16].

1.5.1 Mode of action of transcriptional factors

Transcription factors act through different mechanisms including:

A. Stabilize or block the binding of RNA polymerase to DNA

B. Catalyze the acetylation or deacetylation of histone proteins. The transcription factor can either do this directly or recruit other proteins with this catalytic activity. Many transcription factors use one or the other of two opposing mechanisms to regulate transcription [17]. Histone acetyltransferase (HAT) activity – acetylates histone proteins, which causes

the association of DNA with histones to be weak, and make the DNA more accessible to transcription, leading to the up-regulation of transcription. Histone deacetylase (HDAC) activity – deacetylates histone proteins that cause strength of the association of DNA with histones, and make the DNA less accessible to transcription, resulting in down-regulating transcription.

C. Recruit co activator or co repressor proteins to the transcription factor DNA complex [18].

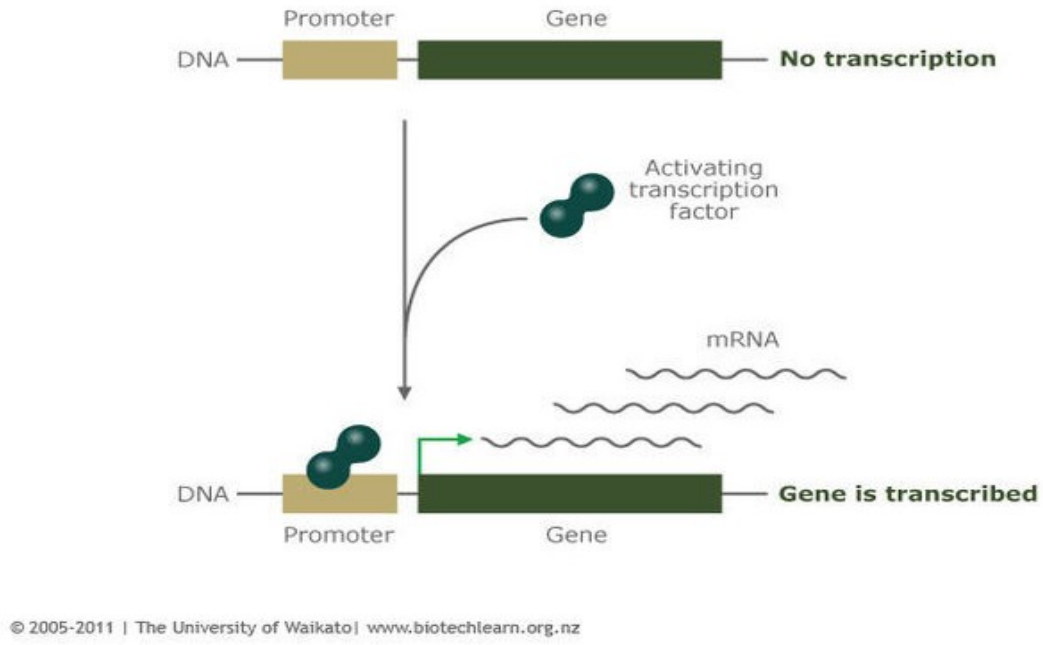

Figure 1.2 Transcription factor binding to DNA [18].

1.5.2 Biological role of transcriptional factors

The main function of transcription factors, which are considered as one of the groups of proteins that read and interpret the genetic “blueprint” in the DNA, are to help initiate a program of increased or decreased gene

transcription, they are vital for many important cellular processes. They are involved in:

1.5.2.1 Basal transcription regulation

General transcription factors (GTFs), are an important class of transcription factors in eukaryotes that are necessary for transcription to occur [19, 20]. Many of these GTFs don't actually bind DNA but are part of the large transcription pre-initiation complex that interacts with RNA polymerase II directly. The most common GTFs are TFIIA, TFIIB, TFIID, TATA binding protein, TFIIE, TFIIF, and TFIIH [21]. The binding of the preinitiation complex to promoter regions of DNA is upstream to the gene that they regulate.

1.5.2.2 Differential enhancement of transcription

Regulation of the expression of various genes is by binding of the transcription factors to enhancer regions of DNA adjacent to regulated genes. These transcription factors are critical for making sure that genes are expressed in the right cell at the right time and in the right amount, depending on the changing requirements of the organism [22].

1.5.2.3 Development

Many transcription factors are involved in development mainly in multicellular organisms [22]. When there is a stimulus these transcription factors turn on/off the transcription of the appropriate genes, which, in turn, allows for changes in cell morphology or activities needed for cell fate determination and cellular differentiation. For example, the Hox

transcription factor family is important for proper body pattern formation in organisms as diverse as fruit flies to human's response to intercellular signals [23].

Cell communications occur by releasing molecules that produce signaling cascades within another receptive cell. If the signal requires up regulation or down regulation of genes in the recipient cell, often transcription factors will be downstream in the signaling cascade [24]. An example of signaling is estrogen, a fairly short signaling cascade that involves the estrogen receptor transcription factor. Tissues such as the ovaries and placenta secrete Estrogen; which crosses the cell membrane of the recipient cell, and is bound by the estrogen receptor in the cell's cytoplasm. The estrogen receptor then goes to the nucleus of the cell and binds to its DNA binding sites, causing the transcriptional regulation of the associated genes to be changed [25].

1.5.2.4 Response to environment

Transcription factors do not only act downstream of signaling cascades related to biological stimuli but they can also be downstream of signaling cascades involved in environmental stimuli. For example heat shock factor (HSF), which up regulates genes necessary for survival at higher temperatures, other examples include the hypoxia inducible factor (HIF), which up regulates genes necessary for cell survival in low-oxygen environments, and the sterol regulatory element binding protein (SREBP), which helps to keep the proper lipid levels in the cell [26].

1.5.2.5 Response to environment

Oncogenes or tumor suppressors are examples of transcription factors that help regulate the cell cycle and as such determine how large a cell will get and when it can divide into two daughter cells [27]. Other example is the Myc oncogen, which has important roles in cell growth and apoptosis).

1.5.2.6 Cell cycle control

Alteration of gene expression in a host cell is also done by transcription factors to promote pathogenesis. The best examples of this are the transcription-activator like effectors (TAL effectors) which is secreted by *Xanthomonas* bacteria. Following its injection into plants, these proteins can enter the nucleus of the plant cell, bind plant promoter sequences, and activate transcription of plant genes that aid in bacterial infection [28]. TAL effectors contain a central repeat region in which there is a simple relationship between the identity of two critical residues in sequential repeats and sequential DNA bases in the TAL effecter's target site [28, 29]. This makes it easier for these proteins to evolve in order to better compete with the defense mechanisms of the host cell [30].

1.5.2.7 Pathogenesis

Transcription factors are the ultimate targets of cell-signalling pathways. Whenever cells need to response to an extracellular signal such as a hormone, the response is mediated by a change in gene expression that comes about, most often as the result of a change in the phosphorylation state of a transcription factor. Transcription factors not only do control the

rates of transcription to regulate the amounts of gene products (RNA and protein) available to the cell. But transcription factors themselves are regulated often by other transcription factors.

1.6 The ETS family of transcription factors

The ETS family of transcription factors plays important roles in biological processes and known to act as positive or negative regulators of the expression of genes including those that control response to various signaling cascades, such as proliferation, differentiation, development, transformation, apoptosis, migration, invasion and angiogenesis in various cell types such as B cells, endothelial cells, fibroblasts as well as in different neoplastic cells [31, 32, 33, 34, 35]. In humans, the ETS family consists of 27 members characterized by an evolutionary highly conserved DNA-binding domain, the ETS domain,which consist of 80 amino acids with 4 tryptophane repeats [36]. Rearrangements in several genes of the ETS family have been reported in prostate cancer [37]. Translocations between the TMPRSS2 gene, an androgen-regulated prostate-specific serine protease, with several members of the ETS family such as ETV-1, ETV-4, ETV-5, and most commonly ERG, are frequently reported in human prostate cancers [35, 37, 38].

The consequences of these translocations seem to result in increased expression of the rearranged ETS-factors as a response to androgens [35]. Based on phylogenetic analysis of the ETS domains, subfamilies of more highly related members were identified, and the DNA-binding properties of ETS proteins were found to be similar due to the amino acid conservation within the ETS domains [39]. Nonetheless, it has been suggested that the

ETS proteins have unique biological functions, and that different ETS proteins may exhibit preference for distinct flanking sequences that could facilitate specificity. Furthermore, redundant occupancy of the ETS family members at proximal regions and overlapping functions for ETS family members has been reported. It has been suggested that the ETS family members show distinct but partially overlapping expression patterns and achieve specificity via cooperative binding to other transcription factors **[**35].

In order to understand the role of the ETS family members in prostate cancer, the expression profile of the entire ETS family members in different prostate cancer cell lines and tissues has been reported. The findings indicate an insight into the expression specificity of the various ETS family members in these cell lines [40].

Additionally, the above study has provided the first evidence that ETS family members can regulate each other, by demonstrating that the family prototype, Ets-1, regulates five out of the 27 family members Elf-1, Elf-2.Elk-1,Etv-5 and Spi-1 in PC3 prostate cancer cells [40].

1.7 Aim of study

To understand the cooperative interactions among different ETS family members of transcription factors in prostate cancer, specifically, through the knock down the ETS members ETS-1 and ETS-2 using prostate cancer cell lines PC3 and DU-145, respectively.

Chapter Two

Material and Methods

2.1 Cell culture

DU-145 and PC3 cells were grown in RPMI and F12K media, respectively. The media was suplemented with 0.5% pencellin/ streptomycin and 10% heat inactivated fetal calf serum (Invitrogen, USA). Furthermore, the RPMI media was additionally suplemented with 1% glucose, 1% sodium pyruvate and 1% non-essential amino acids (NEIAA) all chemicals were purchased from Invitrogen.

2.2 Cell harvesting and counting

DU-145 and PC3 cell lines were were maintaind in glucerol in liquid nitrogen. Cells transfered from liquid nitrogen to fresh media and then incubated in CO_2 incubator till a noticible cell density was observed. For harvesting and counting purposes, the following steps were used:

1. Two flasks representing the DU-145 and PC3 cell lines were examined under high power using inverted microscope in order to determine cell density, contamination and viabilty.

2. Media was discarded and cells were washed using PBS solution

3. After washing, PBS was removed and cells were trypsinized in 2ml 0.25% Trypsin solution for 2 minutes in CO_2 incubator

4. An 8ml of RPMI and of F12K media were added to DU-145 and PC3 cells, respectively. Cells were resuspended in Falcon tubes and centrifuged for 3min at 7000rpm

5. Supernatent was discarded and 1ml of freshly prepared media was added to cells

6. 10µl of cell suspention was aspirated and mixed with 10µl of Trypan blue solution

7. Counting was carried out using Hematocytometer under inverted microscope

8. Cells were then seeded on 12 well plates for 24hr in order to reach a cell confluence of 50-60%

2.3 Gene transfection

2.3.1 ETS-1 and ETS-2 gene knock-down

Small interfering RNA (siRNA), sometimes known as short interfering RNA or silencing RNA, is a class of double-stranded RNA molecules, 20-25 nucleotides in length, that play a variety of roles in biology. The most notable role of siRNA is its involvement in the RNA interference (RNAi) pathway, where it interferes with the expression of a specific gene [41].

ShRNA (short hairpin RNA) is a DNA molecule that can be cloned into expression vectors to express siRNA (19-21nt RNA duplex) for RNAi studies. ShRNA is a short nucleotide sequence ranging from 19-29 nucleotides derived from the target gene, followed by a short spacer region of 4-15 nucleotides (*i.e.* loop) and a 19-29 nucleotide sequence that is the reverse complement of the initial target sequence. Figure 2 illustrates the biological role of siRNA in gene regulation [41].

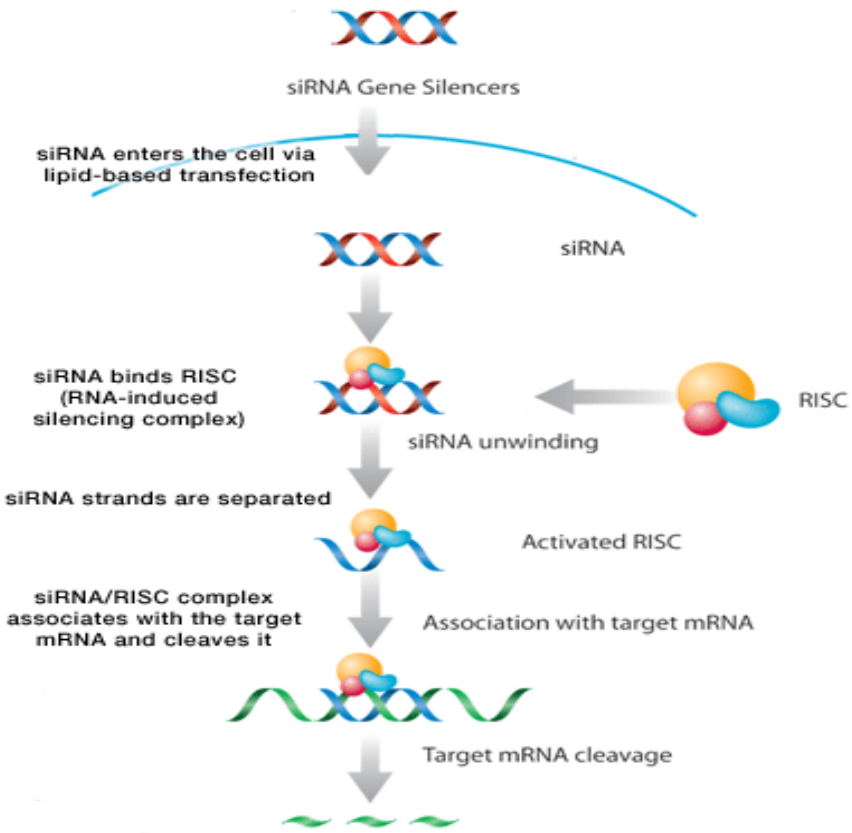

Figure 2.1 siRNA and gene regulation pathway [42].

2.3.2 Transfection: introduction of gene specific shRNA into mammalian cells

ETS-1 and ETS-2 specific shRNA (Origene, USA) plasmids were intoduced into PC3 and DU-145 cell lines, respectively. The process was performed according to manufactorors protocol. In briefe, 6 different plasmids for each of ETS-1 and ETS-2 (members of ETS family transcription factors) were transfected into PC3 (prostate carcinoma cell line of bone origin) and DU-145 (prostate carcinoma cell line of brain origin), respectively. The six plasmids were: four plasmids contain different sequences in the shRNA expression casettes (P1, P2, P3 and P4) and were verified to correspond to the target gene with 100% identity.

The plasmids represent differents sequences of ETS-1and ETS-2 genes (see plate designe and study samples). The other two plasmids were one with a scrambled sequence (Scr) and the other was an emepty vector (negative control).

Figure 2.2 12- well plates.

Transfiction was carried out using the following protocol:

1. Fifty thousand cells of PC3 and 40,000 cells of DU-145 were seeded into a 12 well-plate and incubated overnight in a 5% CO_2 incubator to acheive 50-60% confluence

2. Transfection mixture was prepared in the next day: 1000ng of each plasmid, combined in a small sterile tube with a Serum-free DMEM and a Turbofection 8 solution transfetcion reagent

3. The latter mixture was added to the cells and cells were incubated for 72 hours to achieve transfection perior to RNA extraction.

2.3.3 RNA extraction and cDNA synthesis

After the transfection was verified by the flourocense microscoby, cells were scrabed and prepared for RNA extraction using Qiagen′s RNeasy mini kit (Germany) as described by the manufacture. RNA extraction was performed as follows:

1. Media was removed from the 12 well-plates for cell scrabing
2. Wells were then washed using 1ml of PBS per well
3. 150µl of RLT bffer was then added to each well and cells were scrabed and transfered to the spin tubes - corresponding to the differeft plasmids transfected to cell lines
4. Samples were then centrifuged for 2 min at 3000rpm
5. Cells were then homoginazed in 1 volume of 70% ethanol -150µl
6. The spin column was thrown away (pellted cell depri)

Figure 2.3 Spin tube.

7. A 150µl of 70% ethanol was added to left supernatent and the mixture was transfered to new spin columns

8. Centrifugation was carried for 15s at 10,000 rpm

9. The flow was then discarded and RWI (700µL) buffer was added to pelleted crude RNA extrat in spin column and transfered into new tube.

Figure 2.4. Flow transfer using spin tubes.

10. Repeat step 8

11. A 500µl buffer RPE was added for washing purpose of crud RNA to the Rnaeasy spin column

12. Centrifugation for 2 min at 10,000rpm.

13. Rnaeasy spin column in was placed in a new 2 ml collection tube and the old one was discarded then the flow was thrown and centrifuged at full speed (10,000rpm) for 1 min

14. 30µl Rnase free water was added and mixture centrifuged for 1 min.

15. The spin column was thrown

16. The tube containing RNA extracts was placed on ice till estimation of RNA concentration

17. RNA samples were then stored at -20^{o}C

2.3.4 Measure RNA Concentration

RNA concentration was determined using nano-drop machine. Master mixture for the cDNA synthesis was prepared (extracted RNA, Reaction Mix, and RT Enzyme Mix). The samples were then run on PCR for cDNA synthesis according to manufacturer's specification (Superscript III First-Strand Synthesis Super Mix for qRT-PCR). Figure 2.3 shows the used machines for this purpose.

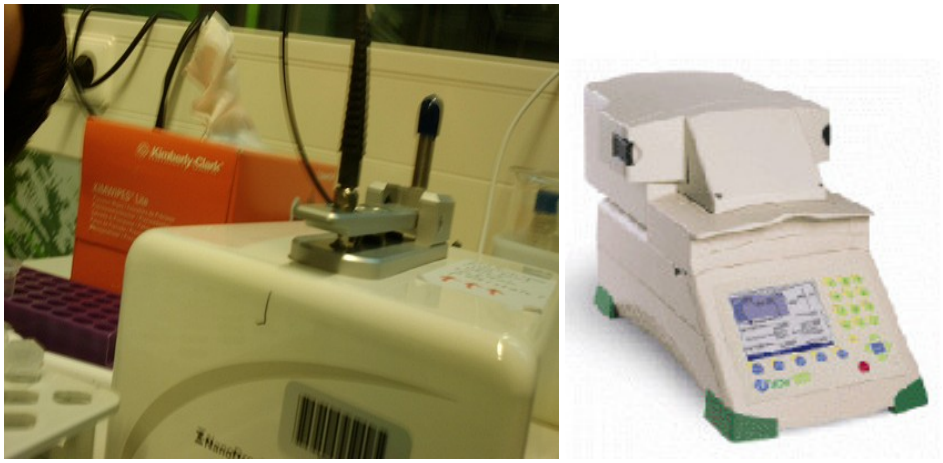

Figure 2.5 Nanodrop machine left side and RT PCR machine right side.

2.3.5 Quantitative real time PCR

cDNA was carried out using previously prepared RNA samples as described in the previous section. For real –time PCR, master mixture was prepared as follows:

1. a total volume of 110µl SYBR-Green ER qPCR Super Mix
2. 1µl of each oligonucleotide primers (forward/reverse). The sequences for ETS-1 and ETS-2 primers were:

ETS-1: p27- forward- CCTCCCCCGGTAAGCTCGG

ETS-1: p27- reverse -TTGGTCCACTGCCTGTGTAG

ETS-2 forward - GAGACGGATGGGAGTTTAAG

ETS-2 reverse – CGTGGTTTGGGATGCAATAAG

3. 2µl DEPEC H_2O

For housekeeping gene, the same procedure was followed as above with the exception of using different oligonucleotides primers. The primers used were:

rpl13a - forward - TACGCTGTGAAGGCATCAAC

rpl13a - reverse - CACCATCCGCTTTTTCTTGT

To 1µl of cDNA samples, a volume of 9 µl of master mixture was added to 384well plates. Samples were analyzed in triplicates. PCR Biosystems 7900HT instrument was used with the following programm: 2 min at 50^{o}C, 10 min at 95^{o}C and 40 cycles of 15 sec at 95^{o}C and for 1 min at 60^{o}C.

Figure 2.6 384 well plates used for qRTPCR.

After 40 rounds of PCR cycls, a melting point analysis was performed with 15 sec 95^{o}C, 15sec 60^{o}C and 15 sec 95^{o}C.

Figure 2.7 qRTPCR machine.

The data were collected after the 60^{o}C step at every cycle and after the melting point analysis. Figure 3.4 shows the main stages of qRTPCR after 40 repeats.

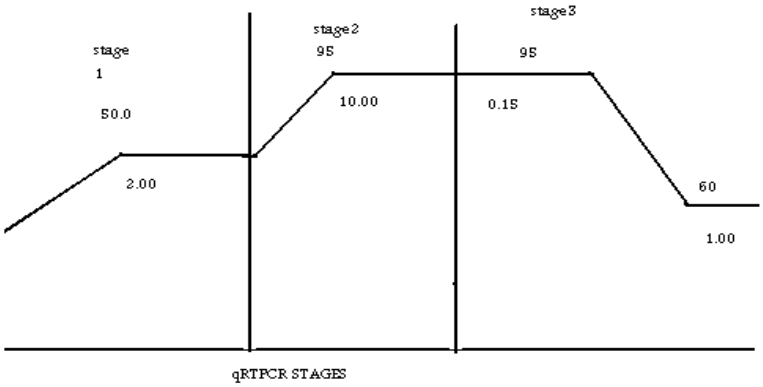

Figure 2.8 Stages of qRTPCR.

Chapter Three

Results and Discussion

3.1 Transfection of shRNA plasmids for (ETS-1 and ETS-2) into prostate cancer cell lines

The choice of PC3 and DU-145 cell lines was based on previous studies where ETS-1 showed high expression among four different selected cell lines (see Figure 3.1) [40]. The relative expression of ETS-1 was measured by quantitative RT-PCR and compared among DU-145, PC3, VCaP and LNCaP prostate cancer cell lines. Accordingly, we decided to knock-down ETS-1 in the PC3 cell lines using shRNA plasmid sequence specific for ETS-1.

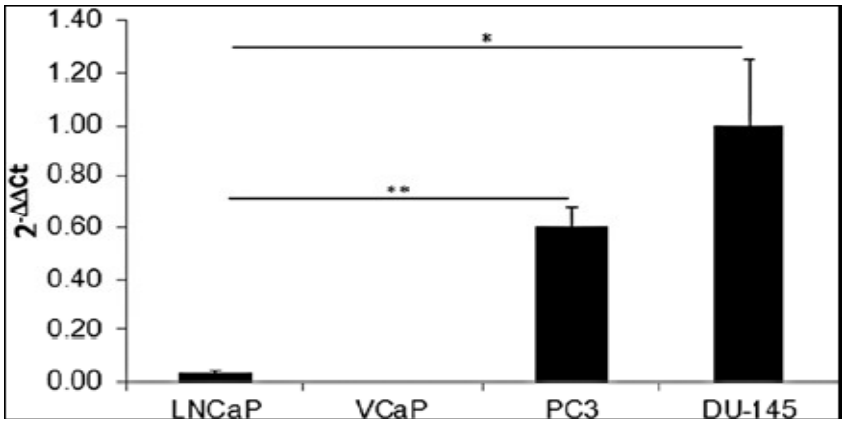

Figure 3.1 Expression of ETS-1 in prostate cancer cell lines. The relative expression of Ets-1 as measured by quantitative RT-PCR was compared among the DU-145, PC3, VCaP and LNCaP prostate cancer cell lines. Significance was calculated with the Student's t-test. **P<0.01 and *P<0.05.[40]

The prostate cancer cell lines PC3 and DU-145 were transfected with green fluorescent GFP ShRNA plasmids that are specific for ETS -1 and ETS - 2, respectively (see Table 3.1 for specific characteristics of the cell lines used). Transfection was confirmed by fluroscence microscopy as shown in Figures 3.2a and b for PC3 and DU-145 cell lines, respectively. Data presented in Figure 3.1 strongly indicates the success of transfection as indicated by green florescent cells. In our experiments, both of the cell

lines used were transfected successfully with all plasmids including scrambled and negative controls. However, the transfection was more pronounced in the PC3 cell line compared to the DU-145.

Table 3.1 Characteristics of the prostate carcinoma cell lines [43].

Cell line	Origin	Androgen responsiveness	Tumourigenicity
PC-3	Human bone metastasis	Androgen independent	High
DU-145	Human brain metastasis	Androgen independent	High

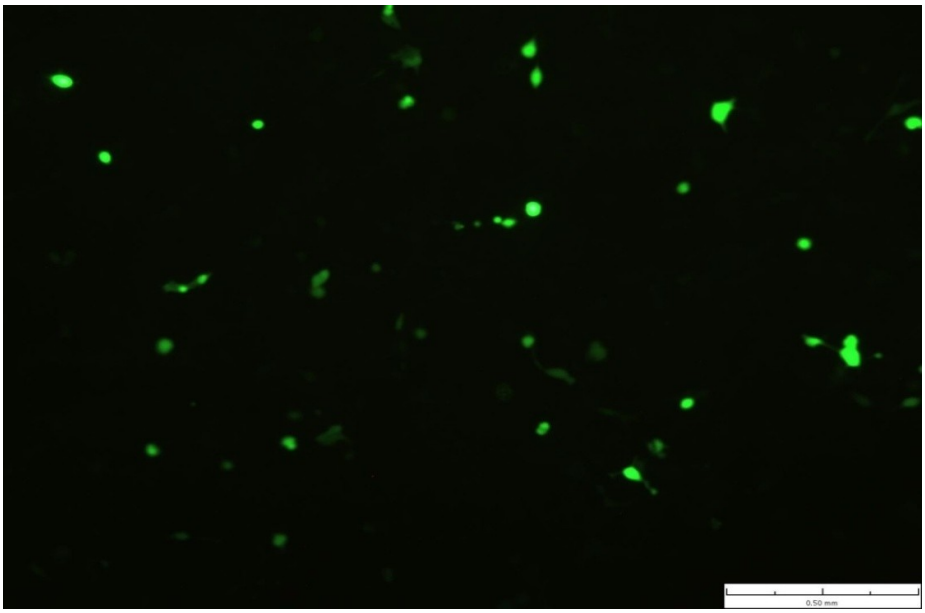

Figure 3.2 a A representative image (100X) of PC3 cell line transfected with shRNA plasmid specific for ETS-1 as observed under a fluorescence microscope.

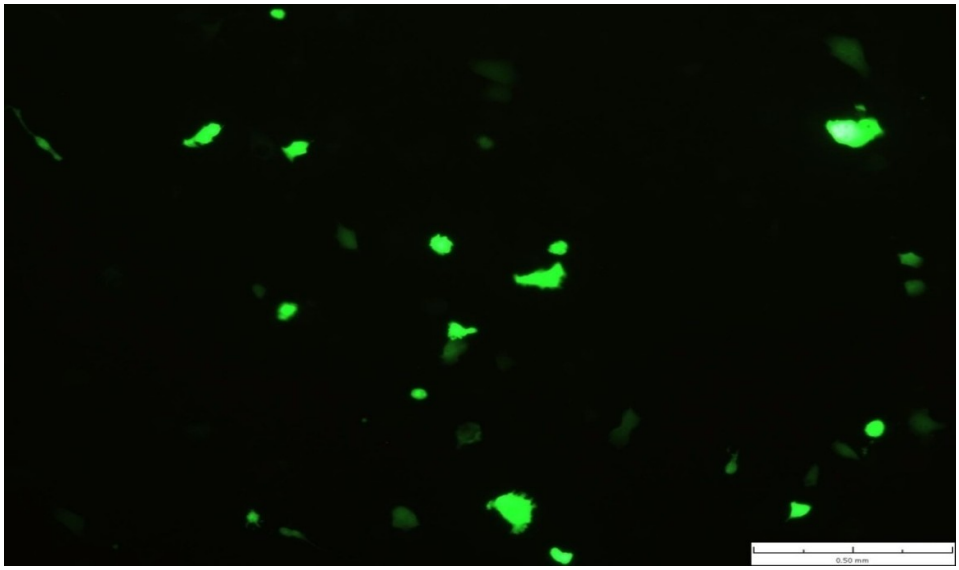

Figure 3.2 b A representative image (100X) of DU-145 cell line transfected with shRNA plasmid specific for ETS-2 as observed under a fluorescence microscope.

3.2 Quantitative Real Time PCR analysis of RNA extracted from prostate cancer cell lines transfected with shRNA plasmids specific for ETS-1 and ETS-2

Data presented in Tables 3.1a and b represent the results of qRT-PCR analysis of RNA extracted from PC3 and DU-145 cells transfected with shRNA plasmids specific for ETS-1and ETS-2, respectively. Absolute Ct-values were assessed with the SDS2.2 software (Applied Bio-systems, USA) and transferred to Microsoft Excel for further analysis. The Ct-values were normalized with the reference to Rpl13a as a housekeeping gene. After that, the $2^{-\Delta ct}$ values were calculated with the values of the scramble control. Significance was calculated with the Student's t-test included in MS Excel. *P*-values are indicated as $^{***}P<0.001$, $^{**}P<0.01$ and $^{*}P<0.05$.

The qRT-PCR analysis shows about 60% knock -down of the *ETS-2* gene by analyzing the data as shown in the figure 3.3. A ***P*** value of 0.020 indicates that the differences between the ETS-2 knock-down and the scramble control is significant.

Since the knock-down of *ETS-2* is only transiently expressed, meaning that the DNA introduced in the transfection process does not usually integrate into the nuclear genome and the foreign DNA will be diluted through mitosis or degraded. Therefore, it is desired that the transfected gene remains in the genome of the cell and its daughter cells; a process requires a stable transfection. Thus, the next step is to create a stable cell line in which the shRNA specific for ETS-2 is integrated into the genome in order to study biological properties of transfected cells such as migration, invasion, and proliferation of the cancer cells.

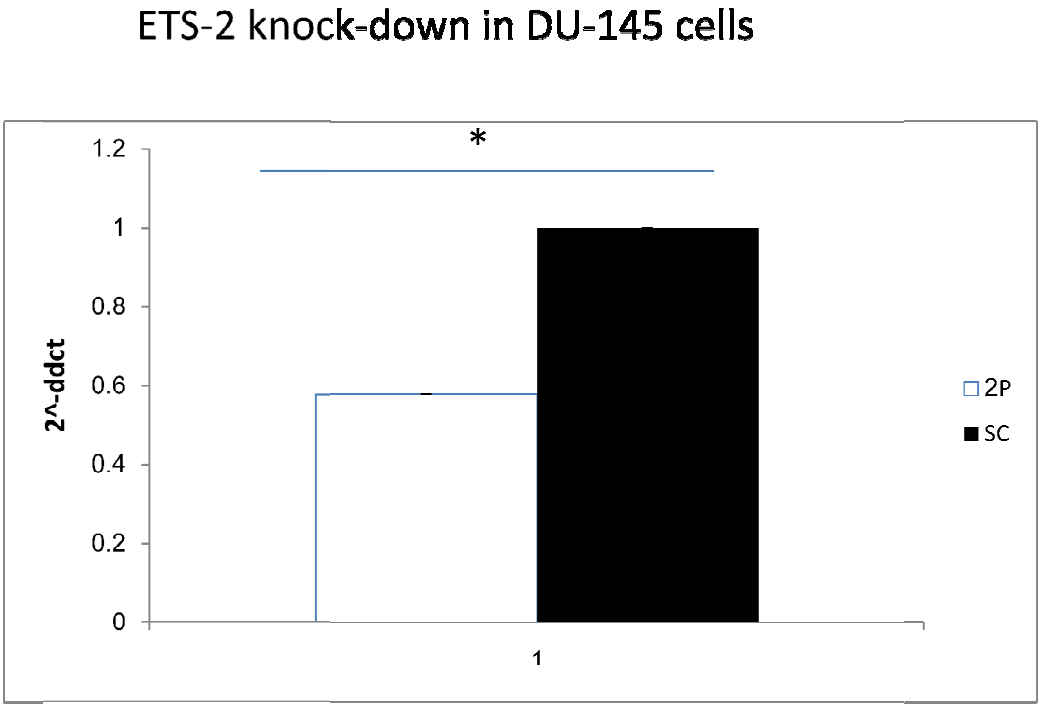

Figure 3.3 ETS-2 knock down in DU-145 transfected with p2 plasmid corresponding to scrambled control; dCT= Ave. CT (ETS-2) – Ave. CT (rIp13); ΔΔCT= dCT- smallest dCT

The qRT-PCR analysis showed no significant differences in the CT-values between the shRNA plasmids (p1, p2, p3, p4) of ETS-1 and the corresponding scrambled and negative control plasmids for ETS -1 gene examined in this study.

qRT-PCR can be used to quantify nucleic acids by two methods, relative and absolute quantification. In this study, we used relative quantification which is based on internal reference genes (housekeeping genes; rpl13a) to determine fold difference in expression of target genes. To quantify gene expression, the measured amount of RNA from the gene of interest is divided by the amount of RNA from a housekeeping gene measured in the same sample to normalize for possible variation in the

amount and quality of RNA between different samples. The cycle at which the fluorescence from a sample crosses the threshold is called the cycle threshold. The quantity of DNA theoretically doubles every cycle, during exponential phase and relative amount of DNA can be calculated 2^n where n represent number of cycles.

The CT of a sample depends on the initial concentration of DNA in the sample, a sample with a lower initial concentration of target DNA requires more amplification cycles to reach the CT, while a sample with a higher concentration require fewer cycles. In general, low CT value indicates high gene expression.

Relative quantification methods correct the sample for differences in quality and quantity like variations in initial sample amount, cDNA synthesis efficiency and or sample loading/pipetting errors, because the quantity of a target and reference gene is a function only of the PCR efficiency and the sample. The normalization procedure is called ΔΔct method and permits comparison of expression of a gene of interest among different samples, so expression of the normalizing reference gene needs to be very similar across all the samples.

The knock -down of the genes examined in this study was not possible and this might be due to several factors:

- Involvement of the genes examined in the RNAi pathway. This is often exploited in experimental biology to study the function of genes in cell culture. Double stranded RNA is synthesized with a sequence complementary to gene of interest and introduced into

cells, where it is recognized as exogenous genetic material and activates the RNAi pathway causing drastic decrease in expression of targeted genes. Decrease in level of expression can indicate the physiological role of the targeted gene products and may not totally abolish expression of the gene (knockdown). In the current study ShRNA was used in the knockdown process. This plasmid is a sequence of RNA that makes a tight hairpin turn that can be used to silence gene expression via RNA interference (see materials and methods for detailed structural composition of this plasmid). The shRNA hairpin structure is cleaved by the cellular machinery in to siRNA which in turn bound to the RNA induced silencing complex (RISC). This complex binds to and cleaves mRNAs which match the siRNA that is bound to it. Thus,if our target gene is involved in the RNA interference pathway this make its knock down impossible.

- Plasmid concentration and transfection efficiency is another problem seems to be associated with failure of knockdown.

- Plasmid design (shRNA) is another important factor that might contribute to transfection and knockdown efficiency. Thus, one should take in consideration that the plasmids used in our experiment might not be optimal for prostate cancer cell lines.

- Protein turn over at the protein level may contribute to the failure of the knockdowns. The fact that even small amounts of a protein can trigger feedback mechanisms and enhance gene expression makes gene knockdown impossible.

Table 3.2a Results of qRT-PCR analysis of RNA extracts for transfected cells of ETS-1

A1	ETS-1 Sonde 2 P1	ETS-1	Unknown	Undetermined
A2	ETS-1 Sonde 2 P1	ETS-1	Unknown	21,102,419
A3	ETS-1 Sonde 2 P1	ETS-1	Unknown	20,501,015
A4	A4			
A5	ETS-1 Sonde2 P2	ETS-1	Unknown	21,001,083
A6	ETS-1 Sonde2 P2	ETS-1	Unknown	2,090,779
A7	ETS-1 Sonde2 P2	ETS-1	Unknown	20,851,858
A8	A8			
A9	ETS-1 Sonde2 P3	ETS-1	Unknown	21,818,562
A10	ETS-1 Sonde2 P3	ETS-1	Unknown	21,954,386
A11	ETS-1 Sonde2 P3	ETS-1	Unknown	21,974,417
A12	A12			
A13	ETS-1 Sonde2 P4	ETS-1	Unknown	21,043,747
A14	ETS-1 Sonde2 P4	ETS-1	Unknown	21,047,426
A15	ETS-1 Sonde2 P4	ETS-1	Unknown	2,087,846
A16	A16			
A17	ETS-1 Sonde2 Water	ETS-1	Unknown	35,223,846
A18	ETS-1 Sonde2 Water	ETS-1	Unknown	Undetermined
A19	ETS-1 Sonde2 Water	ETS-1	Unknown	3,522,027
B1	ETS-1 Sonde2	ETS-1	Unknown	21,195,866

	Neg			
B2	ETS-1 Sonde2 Neg	ETS-1	Unknown	2,149,994
B3	ETS-1 Sonde2 Neg	ETS-1	Unknown	21,529,215
B4	B4			
B5	ETS-1 Sonde2 Scr	ETS-1	Unknown	2,181,157
B6	ETS-1 Sonde2 Scr	ETS-1	Unknown	21,587,513
B7	ETS-1 Sonde2 Scr	ETS-1	Unknown	21,800,219
C1	rpl13a ETS-1 S2 P1	rpl13a	Unknown	1,554,786
C2	rpl13a ETS-1 S2 P1	rpl13a	Unknown	14,775,699
C3	rpl13a ETS-1 S2 P1	rpl13a	Unknown	14,540,326
C4	C4			
C5	rpl13a ETS-1 S2 P3	rpl13a	Unknown	14,799,878
C6	rpl13a ETS-1 S2 P3	rpl13a	Unknown	14,890,204
C7	rpl13a ETS-1 S2 P3	rpl13a	Unknown	15,222,552
C8	C8			
C9	rpl13a ETS-1 S2 P2	rpl13a	Unknown	14,872,838
C10	rpl13a ETS-1 S2 P2	rpl13a	Unknown	14,911,611
C11	rpl13a ETS-1 S2 P2	rpl13a	Unknown	14,763,857
C12	C12			
C13	rpl13a ETS-1 S2 P4	rpl13a	Unknown	14,901,049
C14	rpl13a ETS-1 S2 P4	rpl13a	Unknown	15,120,793
C15	rpl13a ETS-1 S2	rpl13a	Unknown	15,022,445

	P4			
C16	C16			
C17	rpl13a ETS-1 S2 Water	rpl13a	Unknown	Undetermined
C18	rpl13a ETS-1 S2 Water	rpl13a	Unknown	Undetermined
C19	rpl13a ETS-1 S2 Water	rpl13a	Unknown	Undetermined
D1	rpl13a ETS-1 S2 Neg	rpl13a	Unknown	Undetermined
D2	rpl13a ETS-1 S2 Neg	rpl13a	Unknown	14,785,975
D3	rpl13a ETS-1 S2 Neg	rpl13a	Unknown	1,482,365
D4	D4			
D5	rpl13a ETS-1 S2 Scr	rpl13a	Unknown	14,612,635
D6	rpl13a ETS-1 S2 Scr	rpl13a	Unknown	1,475,805

Table 3.2b Results of qRT-PCR analysis of RNA extracts for transfected cells of ETS-2.

M14	Ets2 P1	Et2-2 sonder 2	Unknown	24,082,829
M15	Ets2 P1	Et2-2 sonder 2	Unknown	25,006,765
M16	Ets2 P1	Et2-2 sonder 2	Unknown	25,084,984
M17	M17			
M18	Ets2 P2	Et2-2 sonder 2	Unknown	28,912,605
M19	Ets2 P2	Et2-2 sonder 2	Unknown	28,976,526
M20	Ets2 P2	Et2-2 sonder 2	Unknown	29,295,305
M21	M21			
M22	Ets2 P3	Et2-2 sonder 2	Unknown	25,223,326
M23	Ets2 P3	Et2-2 sonder 2	Unknown	25,912,724
M24	Ets2 P3	Et2-2 sonder 2	Unknown	2,578,489

N1	Ets2 P4	Et2-2 sonder 2	Unknown	25,358,175
N2	Ets2 P4	Et2-2 sonder 2	Unknown	25,808,273
N3	Ets2 P4	Et2-2 sonder 2	Unknown	26,639,822
N4	N4			
N5	Ets2 Neg	Et2-2 sonder 2	Unknown	2,719,391
N6	Ets2 neg	Et2-2 sonder 2	Unknown	27,324,566
N7	Ets2 neg	Et2-2 sonder 2	Unknown	27,589,937
N8	N8			
N9	Ets2 Scr	Et2-2 sonder 2	Unknown	24,647,024
N10	Ets2 Scr	Et2-2 sonder 2	Unknown	2,500,855
N11	Ets2 Scr	Et2-2 sonder 2	Unknown	25,733,555
N12	N12			
N13	Ets2 water	Et2-2 sonder 2	Unknown	Undetermined
N14	Ets2 Water	Et2-2 sonder 2	Unknown	Undetermined
N15	Ets2 Water	Et2-2 sonder 2	Unknown	Undetermined
O1	rpl13a Ets2 P1	rpl13a	Unknown	17,912,868
O2	rpl13a Ets2 P1	rpl13a	Unknown	17,994,524
O3	rpl13a Ets2 P1	rpl13a	Unknown	17,183,424
O4	O4			
O5	rpl13a Ets2 P2	rpl13a	Unknown	21,307,987
O6	rpl13a Ets2 P2	rpl13a	Unknown	2,247,773
O7	rpl13a Ets2 P2	rpl13a	Unknown	22,456,924
O8	O8			
O9	rpl13a Ets2 P3	rpl13a	Unknown	17,804,085
O10	rpl13a Ets2 P3	rpl13a	Unknown	19,970,354
O11	rpl13a Ets2 P3	rpl13a	Unknown	17,736,628
O12	O12			
O13	rpl13a Ets2 P4	rpl13a	Unknown	1,729,254

O14	rpl13a Ets2 P4	rpl13a	Unknown	17,858,747
O15	rpl13a Ets2 P4	rpl13a	Unknown	17,654,129
O16	O16			
O17	rpl13a Ets2 Neg	rpl13a	Unknown	20,646,656
O18	rpl13a Ets2 Neg	rpl13a	Unknown	19,156,454
O19	rpl13a Ets2 Neg	rpl13a	Unknown	2,176,948
O20	O20			
O21	rpl13a Ets2 Scr	rpl13a	Unknown	16,758,347
O22	rpl13a Ets2 Scr	rpl13a	Unknown	16,449,144
O23	rpl13a Ets2 Scr	rpl13a	Unknown	16,983,608

References

1. Berget, S. M., *et al*. 1977. ***Spliced segments at the 5' terminus of adenovirus 2 late mRNA***. **Proceedings of the National Academy of Sciences 74**, 3171–3175

2. Chow, L. T., *et al*. 2007. ***An amazing sequence arrangement at the 5' ends of adenovirus 2 messenger RNA***. **Cell 12**, 1–8

3. Crick, F. 1958. ***Protein synthesis.*** **The Biological Replication of Macromolecules**: **Symposium for the Society of Experimental Biology 12**, 138–162.

4. Stein, J. P., *et al. 1980.* ***Ovomucoid intervening sequences specify functional domains and generate protein polymorphism.*** **Cell 21**, 681–687

5. Parra, G., *et al.* 2006. ***Tandem chimerism as a means to increase protein complexity in the human genome.*** **Genome Research 16**, 37–44.

6. Shilatifard, A., Conaway R. C., Conaway, J. W. 2003. ***"The RNA polymerase II elongation complex".*** **Annual review of biochemistry** 72: 693–715.

7. Karin, M .1990. ***"Too many transcription factors: positive and negative interactions".*** **New Biol.** 2 (2): 126–31.

8. Latchman, D.S. 1997. ***"Transcription factors: an overview".*** **Int. J. Biochem. Cell Biol.** 29 (12): 1305–12

9. Roeder, R. G. 1996. ***The role of general initiation factors in transcription by RNA polymerase II.*** **Trends in Biochemical Science.** 21: 327-335

10. Nikolov, D. B and Burley, S.K. 1997. ***"RNA polymerase II transcription initiation: a structural view".*** **Proc. Natl. Acad. Sci.** 94 (1): 15–21.

11. Lee, T. I. and Young, R. A. 2000. ***"Transcription of eukaryotic protein-coding genes".*** **Annu. Rev. Genet**. 34: 77–137.

12. Mitchell, P. J and Tjian, R. 1989. ***"Transcriptional regulation in mammalian cells by sequence-specific DNA binding proteins".*** **Science** 245 (4916): 371–8.

13. Ptashne, M., Gann, A. 1997. ***"Transcriptional activation by recruitment".*** **Nature** 386 (6625): 569–77.

14. Van Nimwegen, E .2003. ***"Scaling laws in the functional content of genomes".*** **Trends Genet.** 19 (9): 479–84.

15. Babu, M., Luscombe, N., Aravind, L., *et al.* 2004. ***"Structure and evolution of transcriptional regulatory networks"***. **Curr. Opin. Struct. Biol.** 14 (3): 283–91.

16. Brivanlou, A. H and Darnell, J. E. 2002. ***"Signal transduction and the control of gene expression"***. **Science** 295 (5556): 813–8

17. Narlikar, G. J and Fan, H. Y and Kingston, R. E. 2002. ***"Cooperation between complexes that regulate chromatin structure and transcription".*** **Cell** 108 (4): 475–87.

18. Xu, L., Glass, C. K., Rosenfeld, M. G. 1999. ***"Coactivator and corepressor complexes in nuclear receptor function".*** **Curr. Opin. Genet. Dev.** 9 (2): 140–7.

19. Reese, J. C. 2003. ***"Basal transcription factors".*** **Current opinion in genetics & development** 13(2): 114–8

20. Shilatifard, A., Conaway, R. C., Conaway, J. W. 2003. ***"The RNA polymerase II elongation complex".*** **Annual review of biochemistry** 72: 693–715.

21. Thomas, M. C, Chiang, C. M. 2006***. "The general transcription machinery and general cofactors".*** **Critical reviews in biochemistry and molecular biology** 41 (3): 105–78.

22. Lobe, C. G. 1992. ***"Transcription factors and mammalian development".*** **Current topics in developmental biology** 27: 351–83.

23. Lemons, D., McGinnis, W. 2006. ***"Genomic evolution of Hox gene clusters".*** **Science** 313(5795): 1918–22.

24. Pawson, T. 1993. ***"Signal transduction--a conserved pathway from the membrane to the nucleus".*** **Developmental genetics** 14 (5): 333–8.

25. Osborne, C.K., Schiff, R., Fuqua, S. A and Shou, J. 2001. ***"Estrogen receptor: current understanding of its activation and modulation".*** **Clin. Cancer Res.** 7: 4338s–4342s; discussion 4411s–4412s.

26. Weber, L. W, Boll, M and Stampfl, A. 2004. ***"Maintaining cholesterol homeostasis: sterol regulatory element-binding proteins".*** **World J. Gastroenterol.** 10 (21): 3081–7.

27. Wheaton, K., Atadja, P., Riabowol, K. 1996. ***"Regulation of transcription factor activity during cellular aging".*** **Biochem. Cell Biol**. 74 (4): 523–34.

28. Boch, J and Bonas, U. 2010. ***"XanthomonasAvrBs3 Family-Type III Effectors: Discovery and Function".*** **Annual Review of Phytopathology** 48: 419–436.

29. Moscou, M. J and Bogdanove, A. J. 2009. ***"A simple cipher governs DNA recognition by TAL effectors".*** **Science** 326 (5959): 1501.

30. Voytas, D. F and Joung J. K. 2009. ***"Plant science. DNA binding made easy".*** **Science** 326(5959): 1491–2.

31. Oikawa, T. 2004. ***ETS transcription factors: possible targets for cancer therapy.*** **Cancer Sci.** 95: 626-633.

32. Hashiya, N., Jo, N., Aoki, M., *et al*. 2004. **In vivo evidence of angiogenesis induced by transcription factor Ets-1: Ets-1 is located upstream of angiogenesis cascade. Circulation** 109: 3035-3041.

33. Rothhammer, T., Hahne, J. C., Florin A., *et al*. 2004. ***The Ets-1 transcription factor is involved in the development and invasion of malignant melanoma.*** **Cell Mol Life Sci** 61: 118-128.

34. Wernert, N. 2005. ***Ets-1 expression promotes epithelial cell transformation by inducing migration, invasion and anchorage-independent growth.*** **Oncogene** 24: 5384-5388.

35. He, J. Pan Y., Hu J., Albarracin, C., Wu, Y and Dai, J. L. 2007. ***Profile of Ets gene expression in human breast carcinoma.*** **Cancer Biol. Ther.** 6: 76-82.

36. Hollenhorst, P.C., Jones, D.A and Graves, B.J. 2004. ***Expression profiles frame the promoter specificity dilemma of the ETS family of transcription factors.*** **Nucleic Acids Res** 32: 5693-5702.

37. Tomlins, S., Rhodes, D. R, Perner, S., *et al*. 2005. ***Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer.*** **Science** 310: 644-648.

38. Furusato, B., Tan, S. H., Young, D., *et al*., 2010. ***ERG oncoprotein expression in prostate cancer: Clonal progression of ERG-positive tumor cells and potential for ERG-based stratification.*** **Prostate cancer Prostatic Dis.** 13: 228-237.

39. Wei, G., Srinivasan, R., Cantemir-Stone, C. Z, *et al.* 2007, 2009. ***Wide analyses reveal properties of redundant and specific promoter occupancy within the ETS gene family. Genes Dev 21: 1882-1894,***

2007. : Ets1 and Ets2 are required for endothelial cell survival during embryonic angiogenesis. **Blood** 114: 1123-1130.

40. Shaikhibrahim, Z., Lindstrot, A., Langer, B., *et al*. 2011. ***Differential expression of ETS family members in prostate cancer tissues and androgen-sensitive and insensitive prostate cancer cell lines.*** **Int. J. Mol. Med.** 28: 89-93.

41. Hamilton and Baulcombe. 1999. ***A species of small antisense RNA in posttranscriptional gene silencing in plants.*** **Scienc*e*.** 286 (5441): 950–2.

42. RNA interference: ***How do siRNA Gene Silencers work?*** 2011. Santa Cruz Biotechnology Inc. Adopted from: www.scbt. com/gene-silencers. html

43. Kaminski, A., Claus, H. J., Haddouti, M., *et al*. 2006. ***Tumour-stroma interactions between metastatic prostate cancer cells and fibroblasts***. Annette Institute of pathology, University of Bonn.

جامعة النجاح الوطنية
كلية الدراسات العليا

الدور التكاملي لعناصر النسخ في سرطان غدة البروستات

اعداد
جميلة سمير زكي دراغمة

اشراف
د. نائل صدقي محمد ابو حسن
بروفسور د.نيكولاس ويرنيرت

قدمت هذه الأطروحة استكمالاً لمتطلبات درجة الماجستير في العلوم الحياتية بكلية الدراسات العليا في جامعة النجاح الوطنية, نابلس، فلسطين
2011

الدور التكاملي لعناصر النسخ في سرطان غدة البروستات

اعداد

جميلة سمير زكي دراغمه

اشراف

د. نائل صدقي محمد ابو حسن

بروفسور د. نيكولاس ويرنرت

الملخص

تتكون عائلة عوامل النسخ ETS في الأنسان من سبع وعشرون عاملا امتازت بوجود مواقع ارتباط ثابتة على المادة الوراثية DNA. تفضل بروتينات عوامل النسخ هذه التسلسلات الطرفية في التنظيم الجيني والوظائف البيولوجية، وتتدخل أفراد عوامل النسخ لهذه العائلة في العديد من العمليات الحيوية البيولوجية في كل من حالات الخلايا الطبيعية والسرطانية. ان الاختلافات في التعبير الجيني للعوامل المختلفة لهذه العائلة وباستخدام أنماط خلوية مختلفة يعكس صفات خلوية محددة في أنماط الخلايا السرطانية لغدة البروسنات تحديدا. نظرا للتشابه في القدرة المختلفة على الارتباط مع المادة الوراثية DNA وكذلك اعادة الترتيب الجيني بين أفراد هذه العائلة نفسها وجينات مجاورة أخرى من مناطق جينية أخرى يبدو من الصعب تحديد العلاقة التنظيمية بين أفراد هذه العائلة. بينت الدراسات أن الاختلاف في التعبير الجيني لأفراد هذه العائلة في الخلايا السرطانية لغدة البروستات الحساسة والغير حساسة لهرمونات الاندروجين أن العنصر ETS-1 قادر على تنظيم خمسة عناصر من هذه العائلة باستخدام الطرق التقليدية في عمليات النسخ الجيني. عمدت الدراسة الحالية على المحاولة للتخفيف من التعبير الجيني لكل من العناصر ETS-1, ETS-2 باستخدام نمطين خلويين مختلفين من الخلايا السرطانية لغدة البروستات PC3 وDU-145 وذلك باستخدام تقنية البلازميد shRNA في محاولة الحصول على تخفيض دائم في التعبير الجيني لهذه الجينات للتمكن من دراسة ألية عملها في النظام الحي.

لقد تم وبنجاح ادخال البلازميد الى المادة الوراثية للأنماط الخلوية المستخدمة وتبين من تحليل النتائج باستخدام تفاعل البلمرة المتسلسل الكمي الحقيقي qRTPCR أنه من الصعب احداث تأثير على التعبير الجيني للجين ETS-1 على افتراض ان هذه الجين يمكن أن تكون

جزءا من العملية التنظيمية للتعبير الجيني RNAi أو نظرا لتدخل أليات التغذية السلبية الراجعة للبروتينات في الخلايا. اما فيما يتعلق بجين ال ETS-2 فقد تم وبنجاح تحقيق التخفيف من التعبير الجيني بشكل مؤقت داخل الخلايا وبناء عليه اصبح من الضروري البحث في امكانية تخفيف التعبير الجيني وبصفة دائمة داخل الخلايا في النظام الحيوي.