EFFECTIVENESS OF MEISi-2 TREATMENT IN PROSTATE CANCER CELL LINES

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ABSTRACT

EFFECTIVENESS OF MEISi-2 TREATMENT IN PROSTATE CANCER CELL LINES

MEIS proteins are histrocally associated with tumorigenesis, metastasis and invasion in cancer. MEIS and associated PBX-HOX proteins may act as tumor suppressors or oncogenes in different cellular settings. Their expressions tend to be mis-regulated in various cancers. Bionformatical analyses suggest their upregulation in leukemia/lymphoma, thymoma, pancreas, glioma, and glioblastoma, and downregulation in cervical, uterine, rectum and colon cancers. However, every cancer type includes, at least, a subtype with high MEIS expression. In addition, studies highligh that MEIS proteins and associated factors may function as diagnostic or therapeutic biomarkers for various diseases. In this study, the function of MEIS1-2-3 proteins in prostate cancer (PCa) cell lines was investigated *in vitro*. In addition, MEIS inhibitors (MEISi-2), the first in its class, the MEIS homeodomain inhibitor has been shown to have a potent chemotherapeutic agent potential in PC-3, DU145, 22Rv-1 and LNCaP, prostate cancer cell lines. The effects on MEIS inhibitors in PCa cell lines are that reveal cytotoxicity and reduce cell viability. Also, intracellular ROS levels and MEIS protein expression were upregulated post MEISi-2 treatment. Also, MEISi-2 treatment resulted in slightly increase of Pca cell migration and the alteration of cell cycle of Pca cell lines. Overall, it is the first time that Pca cell lines necessitate basal MEIS1/2/3 expression for survical *in vitro*.

ÖZET

PROSTAT KANSERİ HÜCRE HATLARINDA MEISi-2 TEDAVİSİNİN ETKİNLİĞİ

MEIS proteinleri, kanserde tümör oluşumu, metastaz ve invazyon ile tarihsel olarak ilişkilidir. MEIS ve ilişkili PBX-HOX proteinleri, farklı hücresel ortamlarda tümör baskılayıcılar veya onkojenler olarak işlev görebilir. Ekspresyonları çeşitli kanserlerde yanlış düzenlenme eğilimindedir. Biyoinformatik analizler, bunların lösemi / lenfoma, timoma, pankreas, glioma ve glioblastomda yüksek regülasyonunu ve servikal, uterus, rektum ve kolon kanserlerinde düşük regülasyonu öne sürmektedir. Bununla birlikte, her kanser türü, en azından yüksek MEIS ekspresyonu olan bir alt tip içerir. Ek olarak, çalışmalar MEIS proteinlerinin ve ilgili faktörlerin çeşitli hastalıklar için tanısal veya terapötik biyobelirteçler olarak işlev görebileceğini vurgular. Bu çalışmada, MEIS1-2-3 proteinlerinin prostat kanseri hücre hatlarındaki işlevi *in vitro* olarak araştırılmıştır. Ek olarak, sınıfında ilk olan MEIS homeodomain inhibitörü MEISi-2'nin güçlü bir kemoterapötik ajan potansiyeline sahip olduğu gösterilmiştir. Bu çalışmada, MEIS1-2-3 proteinlerinin prostat kanseri (PCa) hücre dizilerindeki işlevi *in vitro* olarak araştırılmıştır. Ek olarak MEIS inhibitörlerinin (MEISi-2), sınıfında ilk olan MEIS homeodomain inhibitörünün PC-3, DU145, 22Rv-1 ve LNCaP, prostat kanseri hücre hatlarında güçlü bir kemoterapötik ajan potansiyeline sahip olduğu gösterilmiştir. PCa hücre dizilerindeki MEIS inhibitörleri üzerindeki etkiler, sitotoksisiteyi ortaya çıkaran ve hücre canlılığını düşüren etkilerdir. Ayrıca hücre içi ROS seviyeleri ve MEIS protein ekspresyonu, MEISi-2 tedavisinden sonra yukarı regüle edildi. Ayrıca MEISi-2 tedavisi, Pca hücre göçünde hafif artış ve Pca hücre hatlarının hücre döngüsünde değişiklik ile sonuçlandı. Sonuç olarak, ilk kez Pca hücre hatlarının hayatta kalabilemek için bazal MEIS1 /2/3 ekspresyonuna ihtiyaç duyduğu gösterildi.

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°C	Centigrade
ACC	Adrenocortical carcinoma
AML	Acute myeloid leukemia
BLCA	Bladder urothelial carcinoma
BRCA	Breast invasive carcinoma
BSA	Bovive serum albumin
CAFs	Cancer-associated fibroblasts
CESC	Cervical squamous carcinoma and endocervical adenocarcinoma
COAD	Colon adenocarcinoma
CRISPR/Cas9	Clustered regularly interspaced short palindromic repeats
DCFDA	5-(and-6)-carboxy-2',7'-dichlorofluorescein diacetate
DLBC	Lymphoid neoplasm diffuse large B-cell lymphoma
DMSO	Dimethyl sulfoxide
EOC	Epithelial ovarian cancer
ESCA	Esophageal carcinoma
FBS	Fetal bovine serum
FDG	Fluorodeoxyglucose
FLI1	Friend leukemia integration 1
FOXP3	Forkhead box P3
GBM	Glioblastoma multiforme
GSCC	Gingival squamous cell carcinoma
HD	Homeodomain
HGSOC	High-grade serous ovarian cancer
HIF	Hypoxy inducible factor
HNSC	Head and neck squamous cell carcinoma
HOTAIR	HOX antisense intergenic RNA
HOX	Homeobox

HPIP	Hematopoetic PBX interacting proteins
hPSCs	Human pluripotent stem cells
IC50	Half maximal inhibitory concentration
KICH	Kidney chromophobe
KIRC	Kidney renal clear cell carcinoma
KIRP	Kidney renal papillary cell carcinoma
LAML	Acute myeloid leukemia
lncRNAs	Long non-coding RNA
MAPK	Mitogen-activated protein kinase
Mcam	Melanoma cell adhesion molecule
MEIS	Myeloid ecotropic viral integration site 1
Meox1	Mesenchyme homeobox 1
min	Minute
miRNAs	Micro RNAs
MLL	Mixed-lineage leukemia
mM	Micro molar
MSLN	Mesothelin
NFDM	Non-fat dairy milk
NSCLC	Non-small-cell lung cancer
OSCC	Oral squamous cell carcinoma
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate-buffered saline
PBX1	Pre-B-cell leukemia transcription factor 1
PDAC	Pancreatic ductal adenocarcinoma
PDX1	Pancreatic and duodenal homeobox 1
PI	Propidium iodine
pre-B-cell ALL	Pre-B-cell acute lymphoblastic leukemia
Prep1	Pbx regulatory protein-1
PSA	Penicillin-streptomycin-amphotericin b solution
PVDF	Polyvinylidene difluoride membranes

ROS	Reactive oxygen species
RT PCR	Real time polymerase chain reaction
SDS	Sodium dodecyl sulphate
siRNAs	Small interfering RNA
TALE	Three aminoacid loop extension
TBS	Tris-buffered saline
µL	Micro liter
µM	Micro molar

1. INTRODUCTION

Homeobox (Hox) genes are transcription factors characterized with a conserved 60 amino acid DNA binding domain called homeodomain. They are important regulators of cell fate, development, tumorogenesis, and stem cell function [1]. They finely regulate the organogenesis in non-vertebrates as well as involve in molecular pathways that determine the vertebrate development [2]. Hox gene expression is modulated by nuclear dynamics, transcriptional regulation, long non-coding RNAs (lncRNAs), RNA processing, miRNAs and translational events [2].

The three amino acid loop extension (TALE) are one of the major homeodomain proteins, that include 27 members characterized by MEIS1-3, PBX1-4, IRX1-6, MKX, PKNOX1-2, TGIF1-2, and their pseudogenes [3, 4]. There are three MEIS isoforms that are called MEIS1, MEIS2, and MEIS3 in mammals [5]. Interestingly, expression levels of MEIS isoforms are quite different from one another in each cell [5]. Meis1 gene was first identified in myeloid leukemia using BXH2 mice. Meis1 gene acts as a viral integration site in myeloid leukemia mice [6].

MEIS1 and Pbx regulatory protein-1 (PREP1) compete functionally with each other [7]. MEIS1 overexpression induces tumor formation in PREP1-silenced mouse embryonic fibroblasts [8]. MEIS, HOX, and PBX proteins bind to the DNA as a heterodimer or trimeric structures [9]. MEIS, HOX, and PBX-generated trimeric structures increase the half-life of the complex [10]. PBX proteins modulate cellular signaling pathways including TGFB, NOTCH, and SMAD, and involve in the remodeling of the chromosomes [11]. When PBX transcription factors malfunction, organ failure, and cardiovascular diseases could occur during development [11]. On the other hand, the deletion of Meis1, downregulates p21, p15, p16, and p19arf expression in cardiomyocytes and improves cell cycle [12]. Intriguingly, overexpression of MEIS proteins may cause caspase-dependent apoptosis in some cells [13]. PBX-MEIS1 protein-protein interaction is required for the induction of caspase-3 and caspase-8 dependent apoptosis [13]. MEIS2-PBX heterodimer controls pancreatic and duodenal homeobox 1 (PDX1) expression in the aciner cells of the pancreas [14]. Generally, MEIS3 expression is involved in neural

development [15]. MEIS3 provides β-cell survival by targeting PDK1 (phosphoinositide-dependent protein kinase 1)[16]. However, HoxA9-MEIS1 interaction has an inhibiting effect on apoptosis in Reh human lymphoblastoma cells,HL-60 32Dcl3 NIH3T3 [13] .

Since the expression of Meis isoforms is different in various cell types and tissues, this suggests that Meis proteins expression may have cell-specific outcomes. K.Somers et al. (2016) studied cytotoxicity in different MLL cells and showed the downregulation of MEIS1, HOXA9, c-MYC, and BCL2, and associated caspase-dependent apoptosis [17]. The upregulation of MiR-155, which is a major inducer of MEIS1 pathway, leads to caspase-dependent apoptosis involving the JNK signaling in AML [18]. In addition, transient overexpression of MEIS1 induces caspase-dependent apoptosis in Reh human lymphoblastoma, Jurkat T, HL-60, and 32Dcl3 cells [13]. Although MEIS-dependent apoptosis response was not yet fully elucidated, there is a relationship between MEIS proteins and apoptosis.

MEIS proteins have been studied in developmental biology, stem, and progenitor cells in different cell types and conditions. Using CRISPR/Cas9 technology, MEIS1 has been represented as a significant regulator for the differentiation of human pluripotent stem cells (hPSCs) into functional hematopoietic cells [19]. MEIS1 is essential for normal hematopoiesis as indicated by MEIS1 mutant mice having an internal hemorrhage, liver hypoplasia, and anemia [20]. MEIS1 suppresses the generation of reactive oxygen species (ROS) and induces scavenging by targeting hypoxia inducible factors (Hif-1α and Hif-2α) in hematopoietic stem cells [21]. Thus, MEIS1 protects the hematopoietic stem cells against ROS by maintaining the glycolytic metabolic phenotype [21]. MEIS-PBX heterodimers are indispensable in the neurogenesis [20]. Dopaminergic periglomerular neurons proliferation in the olfactory bulb is provided by MEIS2 via forming a heterodimer with PAX6 and DLX2 [22]. MEIS2-PBX1 heterodimer could also alter the structure of chromatin and thus modulates the activity of the genes. MEIS2-PBX1 heterodimer, for instance, recruits poly-ADP-ribose (PAR) polymerase 1 (PARP1/ARTD1) in order to regulate PARP1 activity on the chromosome [23].

1.1. MEIS AND ASSOCIATED FACTORS IN TUMORIGENESIS

Studies show that MEIS proteins and its cofactors are mis-regulated in various cancers. Studies highlight that MEIS proteins may be diagnostic and therapeutic biomarkers for cancer and other associated diseases. Thus, here, we will discuss function of MEIS and associated factors in solid and non-solid tumors as well as recent therapeutic approaches including small molecules and non-coding RNAs in MEIS-related cancers.

1.1.1. Bladder Cancer

Even though it is known that MEIS partners directly regulate tumor progression along with HOX proteins, a definitive role for MEIS proteins remains to unclear in bladder cancer. HoxA13 and HoxB13 are, for instance, known to had high expression in urinary bladder cancer [24, 25] HOX Antisense Intergenic RNA (HOTAIR) is proposed as a marker in bladder cancer [26, 27]. HOTAIR downregulates the level of microRNA-205, thereby disrupting the balance of H3K4me3 activity in bladder cancer [28]. Under normal circumstances, 11 HOX paralogs in locus C are involved in healthy urogenital development, however, in the case of bladder cancer, these gene family variants are upregulated [29].

Genetic and epigenetic modifications have an important function in carcinoma formation and DNA hypermethylation is among the most common and characterized epigenetic modification in human malignancies. The methylation level is different between the low/intermediate- and high grade-non-muscle invasive bladder cancer (HG-NMIBC) cohorts for HoxA9 and Isl1 genes, and the methylation level of the HoxA9 promoter is significantly reduced in HG-NMIBC [30]. Besides, compared to non-recurrent tumor samples in recurrent and progressive tumor samples Isl1 and HoxA9 are displayed a remarkably high level of methylation [30]. Therefore, parallel methylation of HoxA9/Isl1 in HG-NMIBC could be used as a predicted value for tumor recurrence and progression [30].

Methylation analysis performed with bladder cancer tissue samples has revealed that Meis1 methylation was similar in all samples regardless of their age [31]. Contrary, Meis1 methylation analysis of patients' urine samples of painless hematuria is also showed that Meis1 is a significant predictor for the presence of urothelial cell carcinoma [32]. Therefore, methylation

analysis itself isn't enough to predict Meis1 function in bladder cancer, and thus, further research is needed.

Alternative splicing has a significant function in post-transcriptional regulation of genes as well as cancer development or progression. Polypyrimidine tract binding protein 1 (PTBP1), which is a protein with a function in formation of bladder cancer metastasis, controls MEIS2 and pyruvate kinase (PKM) via transcriptional mechanisms involving alternative splicing or direct binding to specific introns of these mRNA transcripts. [33]. Besides, Meis2 was overexpressed in bladder cancer tissue compared with normal adjacent tissue [33]. Moreover, Meis2 knockdown significantly inhibits the migration and invasion capacities of bladder cancer cells [33].

Clarifying the potential role of MEIS and how it is related to these HOX proteins in bladder cancer will be an important step in determining the molecular mechanism of bladder cancer and developing new therapeutic approaches.

1.1.2. Breast Cancer

Breast cancer is a hormone-dependent malignant cancer. However, estrogen receptor products, estrogen α, estrogen β, and progesterone are known to be activated in breast cancer via estrogen-independent pathways as well [34]. Therefore, a possible relationship between MEIS and TALE family proteins in breast cancer needs to be investigated in both estrogen independent and dependent pathways [34, 35]. In breast cancer, estrogen receptor induces MEIS1 and Forkhead box P3 (FOXP3) upregulation [35]. MEIS1-FOXP3 interaction with positive feed-back mechanism could enhance the expression of cancer-associated genes associated with and under the estrogen receptor pathway [35]. Expression of MEIS1 along with TMEM25 and REPS2 have been suggested to be marker for breast cancer a prognosis [36]. High expression of MEIS1, TMEM25, and REPS2 is a determining factor for evaluating the probability of relapse and survival of breast cancer [36]. Mutation of HOX/PBX/MEIS interactions may also contribute to breast cancer [37]. Interestingly, interactions of HOX/PBX proteins are unstable in the absence of MEIS and their expressions are aberrant in breast cancer [37]. Taken together, these data

indicate that MEIS1 and associated factors may demonstrate an oncogenic function in breast cancer development and progression.

1.1.3. Colorectal and Gastric Cancers

Meis, Hox, genes and Hif-Meis target genes are largely misregulated in colorectal cancer [38]. Distant metastasis and associated mortality are closely related to MEIS2 expression in colorectal cancer [39]. *In vitro* and *in vivo* studies suggest that MEIS2 may inhibit migration, invasion, and the epithelial-mesenchymal transition of colorectal cancer [39]. Besides, high MEIS2 expression may reduce the overall survival period of patients with colorectal cancer [39].

Different HOX proteins are reported to serve for tumor formation, metastasis, lymph node metastasis, and cancer stem cells (CSCs)self-renewal in colorectal cancer [40]. PBX3 protein that forms a heterodimer with MEIS proteins is overexpressed in colorectal cancers [41]. PBX3 protein, in particular, contributes to cell proliferation, invasion, and metastasis in colorectal cancers by modulating MAPK / ERK signaling pathways [41]. The expression pattern of HOXA9 has been found to be more characteristic of colonic adenocarcinomas [42]. HoxA9 mRNA and protein expression were increased in colorectal tumor tissues compared with normal tissues and elevated HoxA9 expression is correlated with lymph node metastasis [43]. In addition, knockdown of HoxA9 and HoxA4 in HT29 cells leads to a significant decrease in cell proliferation and their overexpression causes an increase in the self-renewal ability of colorectal CSCs [44]. Moreover, in colorectal cancer cells, nucleus accumbens-associated protein 1 (NAC1) expression is elevated and it also increases drug resistance by upregulating HOXA9 expression [45]. On the other hand, mir133b downregulates HOXA9 expression that inhibits colorectal tumor cell proliferation and migration [46].

In comparison to MEIS2 and HOXA9, it is shown that MEIS1 is downregulated in colon cancer [47]. For example, the expression level of MEIS1D27, a truncated splicing variant of MEIS1, has been reported to decrease in the primary colorectal cancer samples [47]. This offers that MEIS1D27 could function as a tumor suppressor in colorectal cancer [47]. Moreover, it is shown that BRAFp.V600E- mutation related to methylation of MEIS1. In tumors and colon

cancer cell lines, this situation has been associated with a reduction of both the full-length MEIS1 and a truncated isoform MEIS1D27 transcript expression [48].

Development and metastasis of the esophageal squamous cell carcinoma (ESCC) involve varying and complicated signaling pathways. Rad et al. (2016) showed that in ESCC, expression of MEIS1 has an reverse association with lymph node involvement, metastasis, and tumor staging [49]. Enhancer of zeste homolog 2 (EZH2) leads to MEIS1 down-regulation during ESCC progression [49]. Moreover, expression of MEIS1 is inversely correlated to SOX2 expression in ESCC tumor samples [49]. The inverse correlation between Mastermind Like Transcriptional Coactivator 1 (MAML1) and MEIS1 is shown in ESCC patients as well [50]. On the other hand, a positive correlation between Musashi RNA Binding Protein 1 (Msi1) and MEIS1 has been reported during ESCC progression [51].

MEIS1 may function as a tumor suppressor during the progression of gastric cancer [52]. MEIS1 expression is diminished in gastric cancer tissues or cell lines, however, when it is overexpressed, proliferation, colony formation, and anchorage-independent growth of gastric cancer cells were repressed [52]. In addition, overexpression of MEIS1 could lead to G1/S cell cycle arrest and cell death of gastric cancer cells [52].

1.1.4. Glioblastoma, Glioma, and Neuroblastoma

Glioblastoma and neuroblastoma both originate from nervous system cells [53]. Neuroblastoma is cancer that occurs in the nervous system during the embryonic development while glioblastoma occurs in the adult brain [53]. HOX proteins are highly studied in tumors of the nervous system. Studies done in U-118, U-138, and NHA glioblastoma cell lines showed that HOXC8, HOXC10, HOXD1, HOXD4, HOXD9, HOXD10, and HOXD13 were expressed in glioblastoma cells while they were not detected in healthy ones [54]. HOXC6 and HOXC10 overexpression regulate glioblastoma cell proliferation and migration via MAPK and PI3K / AKT signaling pathways, respectively [55, 56]. HOXA13 directs cancer invasion by WNT and TGF-β pathways [57]. Intriguingly, HOXA10 allows glioblastoma cells to gain resistance to chemotherapic agents [58].

Pbx3 mRNA and protein content was higher in glioblastoma cells, and migration-invasion of glioblastoma cells is triggered by PBX3/MEK/ERK1/2 /LIN28/let-7b [59]. *In vivo* PBX3 suppression resulted in reduced invasion of glioblastoma [59]. Intriguingly, there is still a need to establish the direct role of MEIS proteins in glioblastoma. Given the high levels of expression of MEIS1-2-3 in glioblastoma tissues, a study examining the relationship between MEIS1-2-3 and glioblastoma is clearly needed.

Glioma, one of the most prevalent and heterogeneous tumors of brain, is characterized by high morbidity and death rates [60]. These tumors are thought to originate from the neuroglial stem or progenitor cells [60, 61]. Nuclear receptor SET domain-containing protein-1 (NSD1) silencing by epigenetic modification leads to Sotos syndrome, as well as nonhereditary neuroblastoma and glioma development [62]. Hypermethylation of Nsd1 causes upregulation of MEIS1 transcript and protein due to the absence of NSD1 binding to the MEIS1 promoter in neuroblastoma cells [62]. A high level of MEIS1 transcript has been seen in Sotos syndrome lymphoblastoid cells because of Nsd1 silencing as well [62]. Gene expression analysis of glioma and glioblastoma cells reveals that Meis2 is one of the differentially expressed genes and it could be a prognostic biomarker for glioma and glioblastoma development along with other genes including Meox2, Pitx2, Nr2e1, and Tfap2B [63]. miR-638 modulates HOXA9 expression in glioma cell lines [64]. It is shown that miR-638 expression is related to tumor size and score in glioma. Overexpression of HOXA9 clears tumor suppressor effects of miR-638 [64]. Besides, cyclin D1 and c- MYC are controlled by miR-638 and HOXA9 [64].

Gene expression analysis of spinal cord ependymomas reveals that 105 genes were upregulated [65]. Among them, Arx, HoxC6, HoxA9, HoxA5, and HoxA3 are top-ranked five genes [65]. Another gene expression analysis has shown that HOTAIR is overexpressed in high-grade gliomas and it is likely controlled by DNA methylation along with co-expression of HoxA9 [66]. Therefore, these two genes could be a prognostic marker for high-grade gliomas. Immunohistochemical analysis in patients carrying clinical intramedullary spinal tumors has shown that both HOXA9 and HOXB13 are upregulated in these samples [67]. Moreover, they involve in spinal ependymoma and myxopapillary ependymoma development, respectively

[67]. Taken together, each member of HOX family proteins has unique functions in glioma, thus they could be useful as specific markers of the disease.

Analysis of Hox genes in neuroblastoma started in early 90's [68]. Neuroblastoma cells induced differentiation using retinoic acid that resulted in Hox gene upregulation [69]. The expression levels of HoxC6, HoxD1, and HoxD8 genes were increased significantly by chemical induction while expression of Hox genes cells were undetectable in the undifferentiated neuroblastomas. In addition, the expression of HoxD4 and HoxD9 were detected at low levels in human neuroblastoma cells [70]. HOXC9 may act as a tumor suppressor and upregulated HOXC9 may activate the intrinsic apoptosis signaling pathways in neuroblastoma cells [71].

The TALE homeobox genes are very important for standart development of the nervous system. They are implicated in neuroblastoma, glioma, and glioblastoma. Most TALE subfamilies (including Meis1, Meis2, and Pbx2) are up-regulated in a set of neuroblastoma cell lines, recommending the regulation of TALE transcription is functional in tumorigenesis [62, 72-74] Intriguingly, stable transfection of the dominant-negative variant of Meis1 is generated clones with broken cell proliferation, gain of differentiated phenotype, and elevated contact inhibition and cell death. Similarly, MEIS2 has been shown to be essential for neuroblastoma cell survival and proliferation [75]. In short, MEIS proteins may act as oncogenes in neuroblastoma reviewed in Geerts et. al. (2003). PHNOX2B transcription factor is expressed under the control of MEIS1-PBX1, AP-1, and NF-κB, complexes and shown to be one of the major triggering factors of neuroblastoma [76]. It was first shown in 2001 that MEIS1 was amplified and overexpressed in neuroblastoma cancer cells [73]. Spieker et al. (2001) have shown in serial analysis of gene expression (SAGE) that MEIS1 proteins were overexpressed in 22 out of examined 24 neuroblastoma cell lines [73]. The overexpression of MEIS1 and MEIS2 in neuroblastoma cells is associated with increased tumor progression [72]. The impairment of cellular proliferation, differentiated phenotype, and induced cellular death occurs in the case of dominant negative splice variant of MEIS1 expression in neuroblastoma cells [72]. Inhibition of Meis1 and Meis2 also causes apoptosis in neuroblastoma cells [72]. To sum up, increased expression of MEIS1 and MEIS2 may involve in the promotion of neuroblastoma formation, thus they may acts as an oncogene [72].

1.1.5. Kidney Cancer

MEIS1 suppresses cellular differentiation and induce self-renewal in embryonic kidney malignancies [77].MEIS1, has pivotal function in the fetal liver formation, is rapidly downregulated during *in vitro* differentiation of kidney [77]. Studies showed that miRNA-204 expression in nephroblastomas is reversely correlated with MEIS1 expression [78]. While miRNA-204 is downregulated, MEIS1, and its binding partner PBX2 are upregulated [78]. In kidney cancer, HOX proteins are also aberrantly expressed [79]. When the interaction between HOX proteins (such as HOXA3, HOXA4, HOXA5, HOXA6, HOXA9, HOXB4, HOXB5, HOXB7, HOXC4, HOXC9, HOXD8, HOXD9, and HOXD10) and PBX proteins are disrupted, necrosis and apoptosis may occur in renal cancer cell lines such as CaKi-2 and 769-P [79]. Studies suggest that MEIS1 may functions as a tumor suppressor in the progression of clear cell renal cell carcinoma (ccRCC) since the endogenous expression of Meis1 was reduced in ccRCC cell lines [80]. Furthermore, elevation of MEIS1 expression is significantly blocked ccRCC cells proliferation and apoptosis [80].

1.1.6. Leukamia / Lymphoma

Leukemia is a disorder that the red blood cells and platelets are disabled by an aberrant increase of white blood cells [81]. Meis1 has been identified as one of the primary factors in the formation of leukemia [82]. Mutations in the MEIS-HOX signaling pathway and its downstream proteins have been found to cause mixed-lineage leukemia (MLL) [83]. PU.1 transcription factor cross-talks with the MEIS-HOX signaling pathway in leukemia cells and promotes cell cycle progression and inhibits cell death [83]. In vivo studies show that PU.1 mutation contributes to MLL development by modulating Meis-Hox downstream genes [83]. The MEIS1-PBX and HOX-PBX heterodimer complexes have been shown to occupy promoter regions of leukemia-related genes [84]. The MLL1-WDR5 protein complex allows high expression of Meis1 and Hox by methylation activity on DNA [85]. It was also found that HOX and MEIS1 expression levels could be decreased as a result of inhibition of MLL1-WDR5 protein-protein interaction [85, 86]. The endogenous expression level of Meis1 determines the severity of MLL [87]. In cases where MEIS1 is inactive in fetal liver cells by the mutation, myeloid transformation loses its capacity for differentiation and self-renewal of leukemia stem cells [87].

The effects and molecular mechanisms of MEIS proteins in leukemia are well-studied [82]. In myeloid cells, MEIS1 is found in a trimeric form with PBX2 and HOXA9 [88]. The trimeric complex occupies the region in which PBX2-MEIS1 DNA is bound [88]. In addition, a regulatory feedback loop linked to MEIS1 along with PU.1, SYK, and miR-146a was reported [89]. Myeloid progenitors transformation with MEIS1 and HoxA9 is dependent on SYK induced MEIS1 expression [89]. PBX-MEIS1 heterodimer causes HOXA9 mediated immortality of myeloid progenitors [90]. Intriguingly, MEIS1-PBX2 interaction provokes chemotherapy resistance towards leukemia [90]. Cellular differentiation is induced by MEIS1 and other polycomb group genes in acute myeloid leukemia (AML) patients [91]. Impairment of MEIS1-HOXA4-9 interaction disrupts epigenetic regulation in the chromosome, therefore, AML formation occurs [91].

In pediatric pre-B cell acute lymphoblastic leukemia (pre-B-cell ALL) patients, Meis1 promoter methylation level is different from control groups [92]. Levels of MEIS1 expression is also correlated with white blood count [92]. Moreover pre B-cell ALL patients have E2A-PBX1 chimeric oncoprotein resulting from chromosomal translocation t(1;19) [93]. Translocation results in mutant oncoprotein [93]. E2A-PBX translocation causes inappropriate tissue-specific gene expression in pre-B cell ALL [93, 94].

MEIS1 and MEIS2 interact with HOX and PBX variants in leukemia [95]. The presence of PBX3 and MEIS1 increases HoxA9-induced leukemia [95]. PBX3 enhances the stability of MEIS1 and PBX3-MEIS1 heterodimer triggers the further transcription of Meis1 [95]. The trimeric structure of HoxA9/MEIS1/PBX3 supports the HoxA9 driven leukemia [95]. In addition, MEIS2 is overexpressed in myeloid leukemia mice, this is a conclusion that MEIS1 and MEIS2 may have a parallel function in myeloid leukemia [96, 97].

MEIS1 has been found to be a very important regulator for the differentiation of human pluripotent stem cells (hPSCs) into functional hematopoietic cells [19]. MEIS1 controls hematopoietic differentiation via targeting T-cell acute lymphocytic leukemia 1 (TAL1) and Friend leukemia integration 1 (FLI1) transcription factors [19]. MEIS2 regulates early hematopoietic differentiation in human embryonic stem cells [98]. Deletion of Meis2 impairs endothelial differentiation that leads to disruption of hematopoietic differentiation [98]. Deletion

of Meis2 differentially modulates TAL1, thereby impairs endothelial specification and endothelial to hematopoietic transition [98].

Aberrant expression of TLX1/HOX11 is also driving the T-cell leukemogenesis via MEIS1 and MEIS2 [99]. MEIS1 or MEIS2 and TLX1 are co-expressed and interact with each other [99]. Intriguingly, TLX1 expression was not observed in B-lineage ALL or in primary lymphocytes [99]. Besides, it has been found that MEIS1/HOXA9 deregulation has an important function in the progression of leukemia [100]. MEIS1 mutations lead to blood cells to develop symptoms of leukemia as well as gaining chemoresistance and proliferation of leukemia cells by triggering other MEIS-cofactors (summarized in **Table 1.1.**).

Table 1.1. MEIS proteins and associated factors involved in leukemia / lymphoma

Associated Factors	Components Complex	Associated Disorder	References
PU1	MEIS1 and PBX3	MLL	[83]
HOXA9	MEIS1 AND PBX3	Leukemogenesis	[84]
AML1-ETO	MEIS1	Acute Myeloid Leukemia	[82]
FLT3	MEIS1, HoxA9	Acute Myeloid Leukemia (AML)	[101]
E2A-PBX1	MEIS1 and PREP1	Myeloid Immortalization	[94]
Polycomb Group Genes (PcG)	MEIS1, HOXA4, HOXA9	Acute Myeloid Leukemia	[91]
SYK, PU1 and miR146a	MEIS1, HOXA9	Acute Myeloid Leukemia	[89]
TLX1/HOX11	MEIS1 and MEIS2	T-cell acute lymphoblastic leukemia (T-ALL)	[99]
Translocation	t(10;14)(q24;q11)-HOX11(TCL3)	T cell leukemia	[102]
Misregulated HOX Genes	MEIS1, PBX2, PBX3	Leukemia Stem Cells of MLL	[3]

Aberrant MEIS1 promoter methylation	MEIS1	Pediatric B-cell acute lymphoblastic leukemia	[92]
Impaired (MLL1)-WDR5 interaction	MEIS1, HOXA9	Mixed lineage leukemia	[85]
HOXA9 and Meis1 downregulation	HOXA9 and HOXB8	Myeloid Differentation	[103]
The t(4;11)(q21;q23) translocation	MLL-AF4 fusion gene and increasing Hoxa9- Meis1	ALL	[104]

1.1.7. Lung Cancer

Ectopic expression of MEIS1 has been shown to inhibit cell proliferation in non-small-cell lung cancer (NSCLC) [105]. This was in parallel with the finding that the reduction of MEIS1 expression led to proliferation of NSCLC cells and cell cycle progression [105]. On the other hand, MEIS1, HOXA5, and T-BOX 5 (TBX5) proteins are considered as pathogenicity markers in lung cancer adenocarcinoma [106]. Thus, PBX2 protein, one of the heterodimer partners of MEIS1, has been shown to cause cell proliferation, metastasis, invasion through activating TGF-β, TGF-β-SMAD3 and Sonic hedgehog signaling pathways [107-111]. HOXA5 and p53 work together to play a role in suppressing lung cancer cell invasion [112]. HOXA5 and p53 reduce the level of matrix metalloproteinase-2 (MMP2), thus inhibiting cell invasion [112]. Various HOX proteins (i.e. HOXA1, HOXA5, HOXB5, HOXC8) have significant roles in lung carcinogenesis [112-115]. Cell proliferation, metastasis, cellular survival, chemoresistance are also involved in the utilization of the Wnt/β-catenin signaling pathway in small and non-small lung [112-115]. Furthermore, the differentiation of methylated CpG of HoxA7 and HoxA9 genes compared to healthy controls is a point that needs to be inspected on a molecular level [116].

HIF proteins are involved in protecting cells against action of ROS-dependent apoptosis [117]. In lung cancer, the effect of HIFs has been the subject of studies because of the cancer microenvironment [117]. HIFs are subject to transcriptional activation via MEIS1 protein [117].

Non-small lung cancers have abnormal and elevated HIF-1α expression [118]. Blocking of HIF-1α complexes has been shown to cause increased metastasis and angiogenesis and an increase in cancer cell proliferation [118, 119]. The consumption of fluorodeoxyglucose (FDG) in lung cancer cells demonstrates the relationship between HIF-1α and HIF-2α [120]. Surprisingly, however, cells that have high expression of HIF-2α are more aggressive against radiotherapy and FDG uptake [120, 121]. MEIS1 decreases cell proliferation in small cell lung cancer [105].In summary, MEIS and HOX proteins may have function as a tumor suppressor in lung cancers.

1.1.8. Skin Cancer

In melanoma cells (namely MJT1 cell line), MEIS1 and PBX proteins enhance the stability of the HOX-PBX-DNA trimeric complex [122]. Disruption of this trimeric complex by designed peptide amphiphiles has been shown to slow down the growth of melanoma cells [122]. In addition, the two-stage skin carcinogenesis mouse model have shown that MEIS1 has a pro-tumorigenic (oncogenic) function in development of tumor development and malign transformation [123]. In the metastatic melanoma cell line, both mRNA and protein expression analysis has shown that PBX2 expression is elevated compared to PBX1, PBX3, and PBX4 [124]. PBX2 and HOXB7 form a heterodimer structure and they induce miR-221 & 222 expressions [124]. PBX2/HOXB7 heterodimer together with miR-221 & 222 downregulates c-FOS expression and block apoptosis [124]. HOXB7, which is abnormally expressed in different melanoma cell lines, has been shown to cause an aberrant cell proliferation through induction of basic fibroblast growth factor (bFGF) [125]. Besides, it has been shown that miRNA-196a modulates the expression of target genes (cadherin-11, calponin-1 and osteopontin) by regulating HoxC8 in melanocyte and melanoma cells [126].

PBX1 is downregulated by promyelocytic leukemia zinc-finger (PLZF) protein in melanoma cells [127]. Besides, knockdown of PBX1 by siRNA leads to suppression of cell growth [127]. Moreover, PBX1 is interacting with HOXB7 in melanoma cells and downregulation of PBX1 causes HOXB7 and target genes downregulation including bFGF, Ang-2 and Mmp9 [127]. miR-495 acting as a tumor suppressor by directly targeting and downregulating of PBX3 [128].

Briefly, miR-495 downregulates proliferation, invasion, and colony formation, as well as inducing apoptosis in melanoma tissues and cell lines [128].

Promoter methylation analysis of primary cutaneous melanoma has shown that HoxA9 is hypermethylated in these samples [129]. Moreover, a detailed DNA methylation analysis of all stages of human melanoma reveals that HoxA9 DNA hypermethylation has a function in tumor development compared to benign samples[130].

Taken together, MEIS1 and PBX2 may be considered to have oncogenic properties in skin tumorogenesis. They contribute the stability of the HOX-PBX-MEIS tertiary structure in DNA; thus, they trigger the proliferation of melanoma cells.

1.1.9. Oral Cancer

Studies suggest that HOX-PBX interactions allows survival of oral malignant and squamous carcinoma cells [131]. Following the disruption of HOX-PBX interaction with double active peptide, c-FOS expression increases and apoptosis takes place [131]. PBX1 and Hematopoetic PBX Interacting Proteins (HPIP) cause cell differentiation, proliferation, metastasis and invasion in oral cancer [132]. Misregulated expression of HOXC6 and HOXA10 cluster proteins are involved in proliferation, survival and migration of oral cell carcinoma. Distruption of HOXC6 and HOXA10 gene expression could enhance tumor progression [133, 134].

One of the most prevalent cancers of the oral cavity is squamous cell carcinoma. Expression pattern evaluation of PBX2 (both mRNA and protein) in patients with gingival squamous cell carcinoma (GSCC) reveals that PBX2 is upregulated and could be a useful prognostic marker in these patients [135]. Overexpression of PBX2 is associated with tumor size, stage, and metastasis as well as high expression of the valosin-containing protein (VCP) in GSCC cells [135].

The hypermethylation of Meis1 has been identified in adenoid cystic carcinoma samples by using methylated CpG island amplification and microarray method, but it needs further validation studies [136]. On the other hand, analysis of global methylation status of 24 Hox genes in oral squamous cell carcinoma (OSCC) cell lines has revealed that 12 genes including

HoxA9 are hypermethylated [137]. HoxA9 hypermethylation is also reported on promoter methylation analysis of OSCC patients' tissues [138] and salivary rinses [139] which leads to the growth advantage of the tumor, and an increase in metastasis [140]. Contrary to these findings, it is also reported that HOXA9 is overexpressed in OSCC tissue samples [141]. Moreover, miR-139-5p inhibits cell proliferation, invasion, and migration by directly targetting HOXA9 expression [141]. These findings indicate it needs further investigation with large patient cohorts to make a clear statement about the function of HOXA9 in OSCC development. Pbx2 and hypermethylation of Meis1 and HoxA9, however, may be considered prognostic markers of oral cancer.

1.1.10. Gynecologic Cancers

Gynecologic cancer arises from anywhere of a woman's reproductive organs. They have categorized into six main types including ovarian, cervical, uterine (endometrial-uterine sarcoma), vulvar, vaginal, and fallopian tube cancer [142]. Each of these types of gynecologic cancers has their unique risk factors, development and treatment strategies [142]. Uterine and ovarian cancers are among the most common gynecologic cancers [142].

Ovarian cancer studies show that MEIS1, MEIS2, nuclear and total cytoplasmic PBX1-4 RNA expressions and protein contents are higher than normal tissue in both nuclear and cytoplasmic locations [143]. Besides, MEIS1 has been shown to play a key role in the early stages of ovarian cancer through its involvement and regulation of T-cell chemo-attraction [144]. Studies about MEIS-PBX proteins and their correlation with HPIP suggest that their potential to become as key uterine cancers biomarkers. High HPIP expression correlates with histological grade, lymph node metastasis and relapse of the cancer. It also reduces overall survival in uterine cancer [145, 146]. Similarly, long non-coding RNA HOTAIR transcribed from HoxC locus in DNA in uterine cancers leads to cellular proliferation, metastasis and radiotherapy resistance via the MAPK signaling pathway [147]. In addition, HOTAIR adversely affects tumor relapse and overall survival time [148, 149].

Analysis of PBX1 expression in ovarian tumor samples indicates that PBX1 is upregulated in these samples, and overexpression of PBX1 leads to platinum-based chemotherapy resistance

in patients with ovarian cancer [150]. In addition, MEOX1 is overexpressed in ovarian cancer cells along with PBX1, and the silencing effects of PBX1 are reversed by MEOX1 expression in cell lines [151]. It is also reported that NOTCH3 is transcriptional activator of PBX1 in ovarian cancer [152].

The overexpression of NOTCH3 is correlated with overexpression of Jagged-1 and PBX1b in cervical squamous cell carcinomas, yet there is no established relationship between PBX1b overexpression and survival of the patients [153]. PBX3 is overexpressed in the cytoplasm of cervical cancer cells and tissues and its' expression is related to poor prognosis, tumor diameter, pathological grade, clinical stage lymph node metastasis, invasion depth, and vascular invasion [154]. The function of PBX3 is likely controlled by the AKT signaling pathway in cervical cancer [154]. The miR-526b miRNA is reduced in various cancers including cervical cancer and miR-526b expression leads to a decrease in PBX3 expression in cervical cancer cells compared to healthy cells [155]. Moreover, the PBX3-mediated epithelial-to-mesenchymal transition process (EMT) is associated with invasion and metastasis and inhibited by miR-526b in cervical cancer cells [155].

Gene expression analysis reveals that Pbx1-2 and HoxB1 regulate COL5A2 expression that possibly has a function in the focal adhesion pathway in endometrial cancer [156]. The benign and rare smooth muscle tumor called retroperitoneal leiomyoma is nearly found in only women and carries histopathological features that are very similar to uterine leiomyomas [157]. In a case with retroperitoneal leiomyoma, it is reported that tumor cells have a t(9;22)(q33;q12) translocation which is resulted in a fusion of Ewsr1 and Pbx3 genes [157]. Tumor-specific methylation analysis of HoxA9 in patients with ovarian cancer phase II clinical trials with bevacizumab and tocotrienol chemotherapy reveals that HoxA9 methylation is increased after one cycle of chemotherapy [158]. This is suggesting the idea that HOXA9 could be a possible biomarker for early response to inefficient chemotherapy in ovarian cancer patients [158].

miR-196b whose expression is upregulated in recurrent epithelial ovarian cancer (EOC) leads to invasion of the ovarian cancer cells [159]. HoxA9 is downregulated by binding of miR-196b to 3'-UTR, therefore it could be a possible candidate for anti-miR-196b effect [159]. The aberrantly expressed HOXA9 in patients with epithelial ovarian cancer has no significant

predictive value during first-line platinum-taxane chemotherapy [160]. On the other hand, a series of studies in ovarian cancer cells indicates that adaptation to the peritoneal environment and guidance of different types of stromal cells to reinforce tumor growth are stimulated by HOXA9 [161]. Although, methylation analysis of HoxA9 with large cohorts has shown that HoxA9 is hypermethylated in both high-grade serous ovarian cancer (HGSOC) [162] and primary ovarian cancer patients, [163] the association of hypermethylation with stage, histological types, grade, and ascites couldn't be established [164]. Interestingly, a study in ovarian cancer cell line and normal tissue has shown that DNA methylation of some of the analyzed genes including HoxA9, HoxA10, Mir34B, Prom1, Cables1, Sparc, and Rsk4 have an inverse correlation with their expression level [165]. It is stated that Rsk4, Sparc, and HoxA9 function as an oncogene in ovarian cancer development since they have less promotor methylation correlated with a high-grade tumor [165]. The high expression of HOXA9 in EOC patients induces P-cadherin via Cdh3 gene activation, which in turns leads to intraperitoneal dissemination of EOC [166]. Overexpression of HOXA9 is associated with poor outcomes in EOC patients and HOXA9 regulates activation of cancer-associated fibroblasts (CAFs) by TGF-β2 in EOC cells [167].

The expression of HOXA9 is required for normal cervical physiology [168] and some cervical cancer cells show downregulation of HOXA9 expression, which is modulated by both methylation and HPV infection [169]. Moreover, targeting the human oncogene B-cell-specific moloney murine leukemia virus integration site 1 (BMI-1) in the cervical cancer cell line leads to HOXA9 activation [170]. On the other hand, another study showed that HOXA9 is expressed in both cervical carcinoma cell lines and normal cervical tissues [171].

Global methylation analysis of HoxA9 expression in endometrial cancer tissues and healthy tissues reveals that HoxA9 is hypermethylated in cancer tissues [172] and it is associated with lymphovascular tumor invasion [173]. Moreover, hypermethylation of HoxA9 was also shown in DNA samples isolated from the vaginal tampon of endometrial cancer patients [174].

In sum, studies suggest that MEIS and PBX proteins may function as oncogenes in gynecologic cancers. Interactions between MEIS1 and PBX1-2-3 are involved in the early stages of ovarian cancer. In addition, they are highly expressed in the gynecologic cancers compared to healthy

counterparts. However, when all the results are evaluated, the oncogenic or tumor suppressive role of HOXA9 in gynecologic cancers is inconclusive.

1.1.11. Pancreatic Cancer

MEIS proteins and its partner PBX1 have been shown to co-occupy promoter of keratin 19 during pancreas development [175]. T3M4, a cellosaurus cell line, is known to have a stimulated cell proliferation through formation of HOXB2- A10-PBX Homeodomain heterodimers and associated pancreatic carcinogenesis [122]. When this HOXB2-A10-PBX Homeodomain complex and target DNA interaction is impaired with a specifically designed peptide, cell proliferation significantly decreases [122]. MEIS1 is an important transcription factor that regulates the expression of mitochondrial genes [176]. MEIS1 protein may alter the mitochondrial activity and associated metabolic pathways in pancreatic cancer [176]. Studies showed that expression of mitochondrial genes could be downregulated when MEIS1 siRNA transfected into pancreatic cancer cells [176]. In addition, MEIS proteins could contribute to Warburg effect to facilitate abnormal growth of cells in hypoxic tumor microenvironment via transcactivation of HIFs and cooperation with HOX proteins. HOX proteins are also known to help the survival of cancer cell by activating ERK1/2 signaling pathways and modulating epigenetic pathways in cancer cells [177]. MEIS1 is overexpressed in primary pancreatic ductal adenocarcinoma (PDAC) cells and causes activation of the melanoma cell adhesion molecule (Mcam) that causes cell migration [178]. On the other hand, global gene expression analysis of nonmetastatic pancreatic endocrine neoplasms and metastatic pancreatic endocrine neoplasms reveals that MEIS2 is downregulated in metastatic pancreatic endocrine neoplasms [179].

PBX1 is differentially expressed in inflammation-associated pancreatic stellate cells (PaSCs) compared to tumor-associated PaSCs [180]. MESOTHELIN (MSLN) is upregulated in most of the pancreatic cancers (PCs) and causes miR-198 downregulation via NF-κB-mediated OCT-2 induction [181]. In addition, the inhibition of miR-198 leads to the upregulation of PBX-1 and VCP that causes tumor progression [181]. In pancreatic cancer cells, overexpression of PBX3 is associated with tumor development and miR-129-5p prevents the proliferation and migration of these cells by targeting PBX3 [182].

The lncRNA HOTTIP is upregulated in pancreatic cancer stem cells (PCSCs) and pancreatic cell lines [183]. HOTTIP is an important factor for the maintenance of PCSCs [183]. Binding of HOTTIP to WDR5 induces HOXA9 expression that is also a significant factor for PCSCs maintenance [183, 184]. The mir-210, a hypoxia-inducible miRNA, inhibits tumor growth of pancreatic cancer by targeting HOXA1, FGFRL1, and HOXA9 [185].

To sum up, MEIS, PBX and HOX proteins may fuction as oncogenes in pancreatic cancers. MEIS1 and PBX3 give metastatic capabilities to pancreatic tumors. PBX1 involves in tumor progression. However, MEIS2 demostrate low expression in metastatic pancreatic cancers [179].

1.1.12. Sarcoma

Mesenchymal cells, are able to differentiate various tissue including adipose, muscle, fibrous, cartilage, and bone, could give rise to sarcoma [186]. Therefore, sarcoma is really a complex disease and so far more than seventy types of sarcoma are described [186]. Sarcoma is genetically complicated as well, such that an increase in mutational burden, complex karyotype, translocation, and amplification could be genetic basis of the disease [186]. Ewing sarcoma is one of the most prevalent and overwhelming primary bone cancer that has been seen both in children and adolescents [187]. It is shown that MEIS1 is an important factor for cell proliferation in Ewing sarcoma cells [187]. In addition, in vivo silencing of Meis1 remarkably suppresses xenograft tumor growth [187]. Moreover, it is reported that MEIS1 functions together with EWS-FLI1 in Ewing sarcoma [187]. G-protein coupled receptor 64 (GPR64) expression is increased in Ewing sarcoma [188]. Besides, downregulation of GPR64 leads to downregulation of tumor growth and metastasis as well as upregulation of PBX2, SLIT2, and MBD2 [188].

MEIS1-NCOA2 fusion transcript has been reported in two cases with primary renal sarcoma [189]. The vascular invasion, cellular necrosis, and perinephric fat invasion seen in both cases point out that Meis1-Ncoa2 fusion gene could be malignant [189]. The aggressive soft tissue sarcoma of malignant peripheral nerve sheath tumors (MPNSTs) can occur both sporadically and together with neurofibromatosis type 1 (NF1) [190]. RNAi screening in MPNST cells

reveals that MEIS1 is an important factor (functioning as a potent oncogene) for MPNST tumor development [190]. It is reported that proliferation and cell survival of MPNSTs by MEIS1 occur via P27Kip inhibition [190]. The miR-873 expression is decreased in the osteosarcoma (OS) tissues and cell lines [191]. Downregulation of miR-873 is relavant with tumor size, clinical stage and distant metastasis [191]. On the other hand, HOXA9 expression is upregulated in OS tissues, and the silencing of HoxA9 recover the miR-873 downregulation effects. This is pointing out that HoxA9 is the target of miR-873 in OS [191].

Taken together, MEIS1 and HOXA9 may function as oncogene in sarcoma [187]. Silencing of Meis1 in xenograft model was found to suppress tumor size in sarcoma [187]. Tumor suppressor roles of PBX2 have also been demonstrated in sarcoma [188].

1.1.13. Thyroid Cancer

Vriens et al. (2011) showed that Meis2 expression in the thyroid cancer patients older than 40 is about 2.5 times more [192]. Also, gene ontology analysis of a hundred genes in four classes of thyroid carcinoma including papillary thyroid carcinomas (PTCs), the oncocytic variants of follicular thyroid tumors (OTTs), tumors of uncertain malignant potential (TUMPs) and follicular adenomas (MAs) reveals that HoxA9 is one of the seven gene that is differentially expressed in thyroid carcinomas and is responsible for tumor development [193].

By comparison of gene expression in Msa, K-119, Koa-2, 8305C, Tco1 anaplastic thyroid cancer cell lines and normal thyroid tissue revealed expression of Hox genes maybe a predictive marker in thyroid cancer [194]. While HoxD9 is expressed only in normal healthy tissue, HoxB4 is expressed in anaplastic thyroid cancer cells [194]. HoxB1, HoxD10, HoxC12, and HoxD13 were not detected in normalt hyroid tissue or anaplastic thyroid cancer [194]. In a different study, HoxA13, HoxB13, HoxC13 and HoxD13 genes have been shown to have abnormal expression in adenoma, papillary and follicular thyroid cancer tissues, and this has been confirmed at the protein level [195]. HOXC10 triggers metastasis and invasion in thyroid cancer and its expression of HOXC10 is inversely related to patient survival time [196].

In addition, HOTAIR transcribed from Hox locus has a significant effect on various thyroid cancers [197]. HOTAIR has been upregulated about 80 times in thyroid cancer [197]. HOTAIR

promotes metastasis of thyroid carcinoma cells [198]. When HOTAIR is downregulated, cell proliferation is inhibited [198].

Although it still requires further confirmations, MEIS2 and HOXA9 appear to have oncogenic features in thyroid tumorogenesis. In addition, various Hox isoforms and HOTAIR also have high expression in thyroid cancer cells and tissues. However there is no generalization for all Hox isoforms. Some Hox isoforms also have tumor suppressing properties.

1.1.14. Thymoma

Thymoma is among the most frequently seen subtype of thymic tumors [199]. It is a tumor of mediastinum that is originated from the thymic epithelium [199]. It can occur at any age with the same incidence in both men and women [199]. The upregulation of Meis1 has been shown in mice thymic epithelial cells (TECs) after inducing thymic atrophy by dexamethasone [200]. In addition, it is also shown that Meis1 is essential for the postnatal thymic microenvironment in TECs [201]. Moreover, in-silico gene expression analysis of medullary TECs has shown that Meis1 and Hoxa7 are upregulated [202]. It is reported that PBX2 has important functions in T-cell development at both the postnatal and embryonic stages of mice [203].

We have found that publications about thymoma and analysis of MEIS1-3, PBX1-3, or HOXA9 expression in thymoma were limited. Jia Y, (2018) showed that expressions of Meis1-3, Pbx1-3, and HoxA9 genes were higher than normal tissue. In addition, we analysed previously published gene arrays [204] and determined differentially expressed genes in varios cancers including thymoma in comparision to healty tissue (**Figure 1.1**). This analysis revealed that Meis1-3, Pbx1-3, and HoxA9 genes are highly upregulated in thymoma compare to healthy adult tissues. Further studies involving thymoma cell lines, *ex vivo* tumor samples and in vivo mouse models are still necessary to determine the relationship of these genes with the development of thymoma.

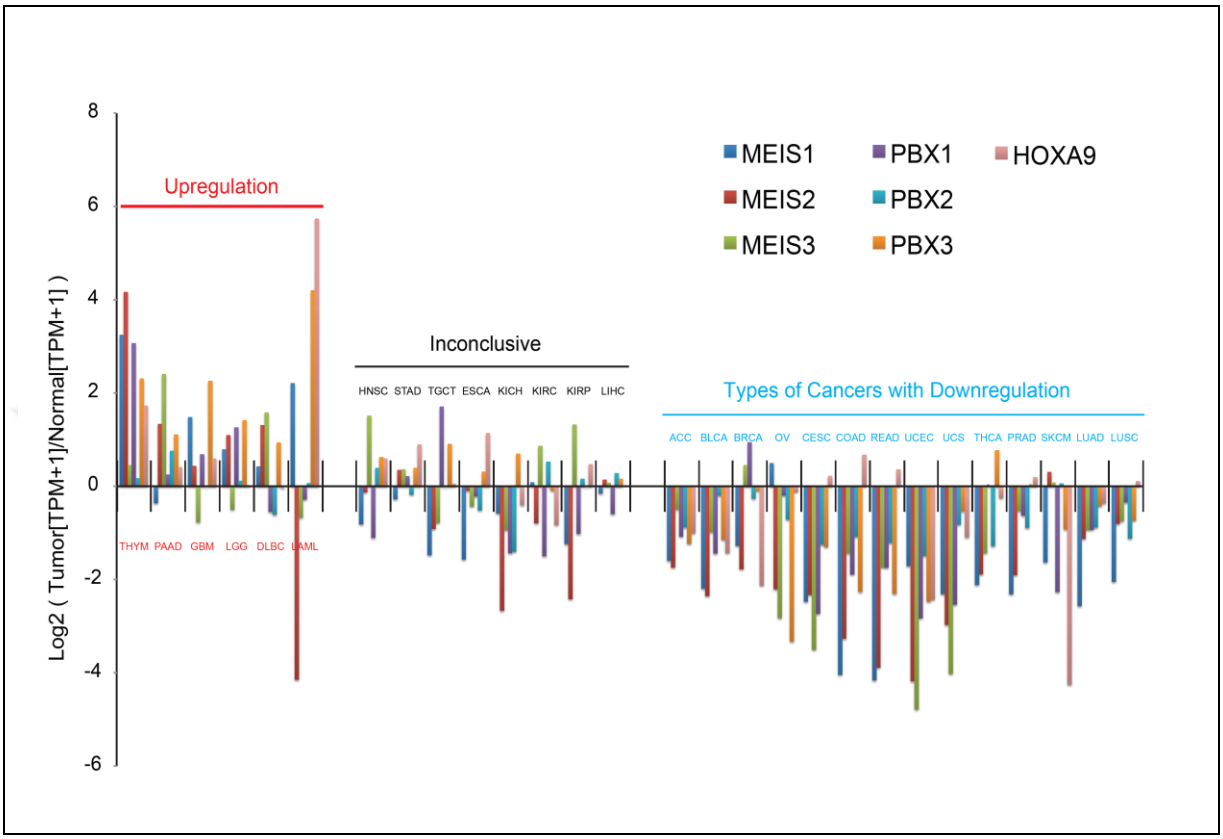


Figure 1.1. Expression profile of Meis1-3, Pbx1-3 and a commonly associated HoxA9 gene in cancer tissues compared to adult healthy counterparts. Cancer types could be separated into three groups based on the overall expression of Meis and its associated factors: Upregulated, inconclusive, and downregulated group of cancers. Note that these are average of all subtypes in the indicated cancers. ACC: Adrenocortical carcinoma, BLCA: Bladder urothelial carcinoma, BRCA: Breast invasive carcinoma, CESC:Cervical squamous cell carcinoma and endocervical adenocarcinoma COAD: Colon adenocarcinoma, DLBC: Lymphoid neoplasm diffuse large B-cell lymphoma, ESCA: Esophageal carcinoma, GBM: Glioblastoma multiforme, HNSC: Head and neck squamous cell carcinoma, KICH: Kidney Chromophobe, KIRC: Kidney renal clear cell carcinoma, KIRP:Kidney renal papillary cell carcinoma, LAML :Acute myeloid leukemia,

1.1.15. Prostate Cancer (PCa)

In healthy individuals, the prostate is an organ with an average weight of 10-12 grams surrounding urethra and located in below urinary bladder [205]. Lobe and zone definitions were made for the prostate structure using histology and medical imaging tools to define the investigations of the prostate structure[206]. Functionally, the prostate is responsible for part of the semen production and is also involved in transporting the eventually formed sperm from the vas defens to the urethra[207].Also prostate secretes prostate-spesific antigen, zinc, fibrinolysin, prostatic acid and phosphatase and some proteolytic enzymes [208].

Pca, one of the frequently diagnosed cancers, is ranked after skin cancer in the men's world. Pca, which is especially seen in elderly men, is a very significant cause of death for this age group [209]. Basicly, pca split four main stages (1-4). In the stage I of prostate cancer, benign located a spesific gland of prostate. In the second stage, pca mass increase and is able to spread both prostate gland. At the stage III, tumor cell can spread close part of body like that seminal vesicles and some lymph nodes. In the final stage, tumor cell can survive without the requirement of hormones. Usually, it is quitely mortal and 5-year survival and survival free metastasis are quitely low than other stages[210].

Historically, HOX proteins known to be related pca progression like other tumorigensis as oncogenic and tumor suppressor roles. Some HOXC genes especially Hoxc8 have oncogenic roles for Pca progression[211]. According to some RT-PCR outcomes, Hoxc4,5,6 and 8 are upregulated malignan pca cell lines and metastatic lymp nodes[212]. Overexpression of HOXC4 and HOXC6 are related with aggresiveteness of pca. The potential mechanism could be related to completion of HOXB13 binding areas[213].In addition HOXC8 can be a major suspicious that is able to suppress androgen receptor induction via SRC-3 recruitment to androgen activity[214].Also, Hoxd13 are upregulated in adenocarcinoma pca compared to untreated controls [215]. The meta-analysis of Hoxa10 show that its role could be tumor suppressor because of the increase of Hoxa10 in pca and Hoxa10 expression inverse correlated with Gleason score[216]. Expression of HOXB7 and HOXA9 tend to trigger metastasis via regulating some angiogenesis factors (angiopoetin-1, FGF2, VEGFA, IL8, eNOS). Also, HOXB7 and HOXA9 causes the alteration of tumor microenvironment. Endothelial cells

increases HOXA9 and HOXD3 expression in order to facilate pca cell angiogenesis.Additionally, HOXA1, HOXA3, HOXA9, HOBX7, HOXC11 and HOXD3 regulate tumor microenvironment for pca, directly or indirectly[217-220]

Mutations in HoxB13 generate a critical risk of developing (PCa) [221]. HOXB13, which enables the co-activation of androgen receptor (AR) and FOXA1, forms heterodimer with MEIS1 [221]. Expression levels of BCHE and TNFSF10 were decreased in PCa cells by MEIS1 [221, 222]. MEIS proteins have been shown to involved in the progression of PCa by modulating c-MYC signaling pathway, cellular proliferation, and associated with invasiveness of PC [223]. Depletion of Meis1 and Meis2 in vivo may cause tumor growth and an increase in the expression of pro-tumorigenic genes c-myc and CD142 [223]. Thus, the expression of MEIS1 and MEIS2 are related to inhibition of metastasis in PCa [223]. Besides, a MEIS cofactor, PBX3, is considered as an important biomarker in aggressive PCa [224].

The androgen and AR have important functions during the development and maintenance of PCa [225]. The potential link between MEIS1 and AR is investigated in PCa cell lines. In the presence of synthetic androgen, MEIS1 prevents the transcriptional function of AR by controlling the translocation of AR from the cytoplasm to the nucleus and inhibits AR binding to the prostate-specific antigen (PSA) gene promoter and enhancer regions as well [225]. In addition, MEIS1 suppresses the proliferation and anchor-independent growth of PCa cells [225]. The hypermethylation and transcriptional downregulation of Meis2 is shown by DNA methylation and RNA expression analyses of PCa tissue samples [226].

Castration-resistant prostate cancer (CRPCa) has a poor prognosis with a challenging treatment [227]. Progression of androgen-sensitive PCa to CRPCa occurs via activation of inflammatory signaling pathway consisting of IκBα/NF-κB(p65), MEIS2, miR-196b-3p, PPP3CC (Calcineurin catalytic subunit γ isoform) [227]. MEIS2 regulates PPP3CC-directed suppression of IκBα/p65/miR-196b-3p pathway, therefore inhibits the development of CRPCa [227]. Gene array analysis of seminal epithelium (SVEC) and normal human prostate epithelium (PrEC) cultures have reported that 15 HOX genes (HoxA13, B2-3, B5-9, B13, C6, D1, D3-4, and D10-11) and their co-factors Meis1 and Meis2 are differentially expressed in PrECas [228]. During

embryogenesis TWIST1 and HOXA9 function together for organogenesis of the prostate and they are overexpressed in PCa cells [229]. The overexpression of TWIST1 leads to migration, invasion, resistance to anoikis and metastasis in PCa cells. HOXA9 expression is regulated by TWIST1 [229]. Additionally, inhibition of HOXA9 is enough to abolish TWIST1-promoted metastasis in PCa [230]. Moreover, while MEIS1, MEIS2, and PBX1 expressions are downregulated, HOXA9 expression is upregulated during tumor initiation and progression of PCa [228]. Therefore, these factors could be useful for both diagnostic and therapeutic purposes [228].

The benign prostate tissue expresses PBX1 and PBX3 in the basal cells nucleus [224]. On the other hand, immunohistochemical staining has revealed that cells are PBX3 positive and PBX1 negative in malign prostate tissue [224]. Moreover PBX3 localization shifts from the nucleus to the cytosol in malign tissue [224]. Furthermore, multiple PBX3 isoforms are reported in PC [224]. Interestingly, patients who present moderate PBX3 staining in PCa cells develop CRPCa earlier than patients with strong staining [224]. PBX1 is downregulated in an androgen-independent prostate cancer cell line that is overexpressing the promyelocytic leukemia zinc finger (PLZF), which is an androgen-responsive gene [231]. Moreover, PBX1 and HOXC8 expression cause androgen-independent growth in PCa cell lines [231]. Interestingly, PBX1 overexpression is also shown in the PCa [232]. Besides, PBX1 leads to both cell proliferation and chemoresistance in PCa [232]. Moreover, the stability of PBX1 is controlled by a deubiquitinase USP9x which decreases the polyubiquitination level of PBX1 [232]. Furthermore, targeting USP9x in PBX1-expressing PCa cells leads to apoptosis [232]. In PCa cells, the expression level of PBX3 is upregulated. This is achieved post-transcriptionally by Let-7d that is an androgen-regulated microRNA and is downregulated in PCa [233].

In the PCa, the high expression of MEIS1 and MEIS2 is needed for the growth of the tumor mass in place [223]. In addition, the expression of MEIS1-2 is associated with the anti-metastatis in prostate cancer [223]. Thus, MEIS1-2 expression determines the aggressiveness of prostate cancer. PBX1-3 and HOXA9, on the other hand, may function as an oncogene in prostate cancer progression.

There are seven conventional therapy for prostate cancer: Watchful waiting or active surveillance, Surgery, Radiation therapy and radiopharmaceutical therapy, Hormone therapy, Chemotherapy, Immunotherapy and Bisphosphonate therapy that prevent losing bone density. However, the conventional therapies are not completely sufficient to cure people with prostate cancer. The importance of the targeted treatment for prostate cancer, therefore, has emerged [234-237].

Long non-coding RNAs are novel target for prostate cancer therapy. HOX transcript antisense intergenic RNA myeloid-specific 1 (HOTAIRM1) have a oncogenic role for pca progression. Due to the reduction of HOTAIRM1 via using siRNA, cell prolifetaion could be reduced in PC-3 cell line and then some apoptotic factor's expression was increased[238]. Additionally, the suppression of HOTAIR caused the inhibition of migration and invasion in DU-145 and PC-3.Bufalin, a major component for tradional Chinese medicine, treatment led to downregulation of HOTAIR and then this reduction caused slow cell proliferation and inhibition of migration and invasion[239].

The germline mutations of HOXB13 is one of the most important factors. Therefore, various studies conducted in order to HOXB13 work in pca cell lines. The result of downregulated HOXB13 pca cell via using a liposome-mediated gene transfer approach, led to the inhibition of cell proliferation due to G1 cell cycle arrest [240]. Circular RNA Itchy E3 ubiquitin protein ligase (Circ-ITCH) is positively correlated with HOXB13. Circ-ITCH are downregulated is some pca samples and then it reveals a tumor suppressor role via regulating HOXB13[241]. On the other hand, some chemotherapeutic combinations against elevated HOXB13 expression in pca provide promising outcomes like that BRD4, a member of the BET bromodomain family, and BET inhibitor (HOTBIN). BRD4-HOXB13-HOTBIN10 was uncovered and then it reveal effective results for CRPCs[242]. Epigenetic regulation of HOXB13 exhibit a potential therapy approach. All-trans retinoic acid (ATRA) is able to inhibit DU145 proliferation via reducing the methylation of HOXB13 especially for androgen negatice pca cell lines [243]. Active androgen receptor (AR) splice variant 7 (AR-V7) have a critical role for pca progression. Also, HOXB13 is able to colocalize with the AR-V7 via interacting HOXB13 physically. The dimerization

promote to up-regulate some target oncogenes. The silencing of HOXB13 diminished the effectiveness of dimerization [244].

Nanoparticle and siRNA combination has emerged as a novel promising chemotherapy method for prostate cancer. GRP78 siRNA and docetaxel combinated each other and then carried via using nanoparticles RGD-PEG-DSPE/CaP that is an effective and biocompatible against pca. This combination provide excellent pharma features like that stability, loading capacity and sustainable in order to inhibir pca cell proliferation [245].On the other hand. Paclitaxel were also used in gold nanoparticle with siRNA. Au110-PEI-PEG5000-AA.siRNA consists a positevely charged anisamide PEGylated and AuNPs and the combination of this delivery system with siRNA mediated-NF-KB pathway uncovered a novel chemotherapeutical approach for pca [246]. Olaparib or rucaparib are conventional Poly (ADP-ribose) polymerase (PARP) inhibitors based small molecules. These small molecules inhibits PARP activity but they do not modulate its expression. Therefore siRNA of PARP was used in order to hinder PARP1 expression.PARP1-siRNA was downregulated some EMT factors expression *in vitro* and in vivo via reducing EGFR and GSK3β (Ser9) in PC-3 cell lines[247].

Kanwal R and her friends reviewed that the importance of miRNAs in pca. Generally, miRNAs is thougth to be their biomarker roles. However, conducted studies showed that some miRNAs should be target for chemotherapy. miR-18a, miR-21, miR-32, miR-96, miR-106a, miR125b, miR-133b, miR-148, miR-181, miR-182-5p, miR-183, miR-210, miR-221, miR-222, miR375, miR-409-5p and miR-429 are known oncegenic features up to now. They are able to regulate cell proliferation hormone dependent or in-dependent, migration/invasion and almost all biological process in pca cells[248].

Emerging roles of small molecules for cancer therapy uncovers the promising approaches. Re-activation of AR is a quitely serious problem at the clinical therapies. 2-(((3,5-dimethylisoxazol-4-yl)methyl)thio)-1-(4-(2,3-dimethylphenyl)piperazin-1-yl)ethan-1-one (IMTPPE) is able to inhibit AR and some its variants against enzalutamide resistance pca cells. In vivo studies indicated that IMTPPE suppress 22Rv-1 tumor progressin in xenograft model[249]. It is known that metastatis stage of pca is really mortal and bone metastatic pca becomes chemoresistance against especially docetaxel. At this point, BKM1972 is likely to be antichemotherapeutic agent

for pca via inhibiting the activity of membrane-bound efflux pump ATP binding cassette B 1 (ABCB1, p-glycoprotein) and survivin that provide chemoresistance [250]. Wnt signal pathway is misregulated in pca cell and mostly required for survival of pca cells. Tankyrase (TNKS) and ubiquitin-specific protease 25 (USP25) stabilization led to pca cell proliferation.C44 breaks down the bond of TNKS and USP25 ant ultimately, the inhibitor led to slow cell proliferation *in vitro*[251]. Most cancer studies showed that ubiquitin ligases are misregulated in pca however, the inhibition approach of ubiquitin ligase remained poor due to lack of catalytic activity and interaction with other proteins. Small-molecule Siah1/2 inhibitors is able to inhibit E3 ubiquitin ligases and then Siah inhibitors reduce colony formation capability, Hif1α mediated pahway and finally Siah2 activity[252]. For ideal pca treatment, the combinational approaches should be essential. Because in the cancer most factors are misregulated simultaneously. Enzalutamide with abiraterone combination is a conventional chemotherapy for pca with the high stage but it efficiency is limited. For CRPC stage, a novel small molecule, PAWI-2 combinated Enzalutamide with abiraterone inhibits cell proliferation pca cells *in vitro* and *in vivo*[253]. Also, a novel small molecule, LG1836, is a canditate as chemotherapeutic agent for CRPC phase. LG1836 can inhibit AR and its variant through provide cytotoxicty for pca cells.LG1836 treatment led to the downregulation of survivin and the promotion of apoptosis[254].

Table 1.2. Role of MEIS protein or its cofactors in various solid cancers

Cancer type	Involvement of MEIS protein or Its cofactors in tumorogenesis	References
Bladder	Meis2 could promote metastasis. HoxA13 and HoxB13 are overexpressed.	[33]
Breast	Meis1 is upregulated in breast cancer	[36]
Colorectal cancers	Meis2 expression is associated mortality in colorectal cancer. Overexpression of Meis1 diminishes cell proliferation.	[47, 255]

Glioblastoma	Overexpression of Pbx3, Hoxa10-13 and Hoxc6-10 causes cell proliferation and chemotherapy resistance	[69, 72-74, 256]
Glioma	Meis1 is overexpressed in Glioma.	[62, 63]
Neuroblastoma	Overexpression of Meis1/2 could induce tumor progression.	[73, 75]
Kidney	Overexpression of Meis1 inhibits renal cell carcinoma cell proliferation.	[80]
Lung	Endogenous Meis1 expression could inhibit cell proliferation	[105]
Skin	Meis1 promotes tumor development. Pbx2 and Hoxb7 determine aggressiveness in melanoma.	[123, 124]
Oral	Hoxa9 is upregulated in oral cancer.	[134]
Gynecologic	Meis1/2 and Pbx1-2-3 are upregulated in gynecologic cancers	[154-156]
Pancreatic cancer	Meis1 and Pbx3 give metastatic capabilities to pancreatic tumors. Pbx1 involves in tumor progression.	[180, 182]
Prostate	Meis1/2 expression determines the aggressiveness of prostate cancer.	[223]
Sarcoma	Silencing of Meis1 in xenograft model was found to suppress tumor size	[187, 190]
Thyroid	Meis2 and Hoxa9 is upregulated in thyroid cancer.	[192, 193]
Thymoma	Meis1-3, Pbx1-3 and Hoxa9 are upregulated in thymoma.	[204]

1.2. COMPARISON OF MEIS EXPRESSION IN VARIOUS CANCER TYPES AND THEIR CORRESPONDING HEALTHY TISSUES

The role of MEIS proteins have been largely studied in embryonic development and cancer progession, however, their function in adult tissues still have not been fully established [257-260]. Several adult tissues demostrate high levels of Mesi expression [261]. Meis1, Meis2 and Meis3 are highly expressed in female reproductive tissues [261, 262]. MEIS1 organizes sex steroid hormones and reproduction with HOXA10 and PBX2 interactions [263, 264]. Adrenal glands have a high MEIS1 expression and followed by adrenal cortex, colon, appendicitis and ovary [262]. Unlike Meis1, Meis2 expression is elevated in the brain, pancreas and prostate [262]. MEIS2 which regulates neurogenesis with co-factors such as PAX6, FOXP1, FOXP2, FOXP3 and PBX3, provides dopaminergic periglomerular fate specification [22, 265, 266]. MEIS3 that is overexpressed in the brain, interacts together with PBX4, HOXb1b and manages the hindbrain fate utilization of the FGF/MAP kinase and PCP signaling pathway [267]. MEIS3 in hindbrain has regulatory roles that is inducing neural progenitor cell and maintaining neural stem cell pool [268, 269].

According to GEPIA and BioGPS datasets, the expression of Meis1, Meis2 and Meis3 have varied in different cancers [262, 270, 271]. Interestingly, Meis1 is overexpressed in lymphoid neoplasm diffuse large B-cell lymphoma, cholangio carcinoma, kidney renal clear cell carcinoma, ovarian serous cystadenocarcinoma, glioblastoma multiforme, acute myeloid leukemia, brain lower grade glioma, and thymoma [262, 270, 271]. Meis2 expression pattern in cancer tissues differs from Meis1 as it differs also in adult tissues. Meis2 has a high expression profile in cholangio carcinoma, glioblastoma, brain lower grade glioma, pancreatic adenocarcinoma, liver hepatocellular carcinoma, skin cutaneous melanoma, stomach adenocarcinoma, and thymoma. The MEIS3 that is the least studied isoforms of MEIS proteins, is highly expressed in breast-invasive carcinoma, diffuse large B-cell lymphoma, kidney renal papillary cell carcinoma, head and neck squamous cell carcinoma, pancreatic adenocarcinoma unlike other isoforms [262, 270, 271]. It is not possible to identify a cancer in which all of the MEIS isoforms are expressed at a high level. As in normal tissues, the expression of MEIS in

cancer is quite different from each other. Only in brain lower grade glioma, the expression of all MEIS isoforms are higher compared to healty counterparts [262, 270, 271].

Another study by Jia Y et al. (2018) demonstratated the profiling of TALE and HOX protein members in cancers reveals a the complex network of MEIS isoforms in cancer biology [204]. The expression of MEIS isoforms have changed in almost all cancer tissues when studied in 28 different cancer types. The Meis1, Meis2 and Meis3 isoforms were upregulated in lymphoid neoplasm diffuse large B-cell lymphoma and thymoma cancers. Meis1 is upregulated in acute myeloid leukemia and glioblastoma. In other cancers, expression of Meis1 is either reduced or do not lead to a significant change. Meis2 and Meis3 expression were increased in pancreatic adenocarcinoma. Meis3, unlike other isoforms, was increased in head and neck squamous cell carcinoma [204].

Overall, we have seen that expression of Meis1-3, Pbx1-3, and associated partner HoxA9 are upretulated in thymoma, pancreatic adenocarcinoma, glioblastoma, glioma, lymphoma and leukemia when compared to corresponding healthy adult tissues (**Figure 1.1**). In particular, Meis1 has been upregulated in breast, neuroblastoma, gynecologic, skin, sarcoma and thymoma cancers; this may be associated with the cancer cells to survive, proliferate and the tumorogenesis. Meis1 and Meis2 have been upregulated in parallel only in neuroblastoma, gynelocologic and thymoma cancers. Meis2 has been shown to induce metastasis in bladder cancer [33]. On the other hand, Meis1 isoforms have tumor suppressor roles in some cancers. Meis1 has a cancer growth suppressor role in kidney and prostate cancer [80, 228]. Meis2 may also function as a tumor suppressor in prostate cancer [228]. In prostate cancer, there is no tumor aggression in patients with high Meis1 and Meis2 expression [223]. Meis1 could trigger cell proliferation in colorectal cancer, while Meis2 determines the relationship between colorectal cancer growth and death [39]. Meis2 could increase colorectal dependent death [272]. In Glioma, Meis1 expression is high, while Meis2 expression is low. Unfortunately, studies on the role of Meis3 protein in cancer biology are very limited. Given the importance of Meis isoforms and cofactors in cancer biology, it is clear that the effects of Meis3 on cancer biology need to be investigated. In fact, the same MEIS isoform along with associated co-factors may have two different functions, tumor suppressor and oncogene in different cancers. This requires further

analysis and understanding of complex intraction of MEIS proteins in different cofactors when to conclude about final outcome of the MEIS activity in the cancer as well as adult tissues.

1.3. NON-CODING RNAs BASED THERAPIES IN CANCER AND MODULATION OF MEIS AND ITS PARTNER EXPRESSION

Non-coding RNAs are involved in various biological processes including regulation of translation, RNA splicing, DNA regulation, gene regulation, genome defense, chromosome structure and may act as a hormone [273]. The expression of non-coding RNAs has been often found to impaired in cancer [273-275]. Non-coding RNAs, especially siRNAs and long non-coding RNAs have potential to be used as chemotherapeutic agents in cancer treatments [276]. siRNAs, which is a double-stranded RNA molecule with a length of 20-25 base pairs, are complementary to the mRNA of the specific gene [277]. Therefore, the expression level of an oncogene can be targeted by limiting the translation level with siRNA treatments [276, 277]. Several siRNAs have been shown to reduce cancer proliferation, metastasis, invasion and impaired cell cycle [277]. In addition, some siRNAs could stimulate apoptosis in prostate cancer, oral squamous cell carcinoma, lung cancer, nasopharyngeal carcinoma, osteosarcoma and others [278-282]

miRNAs could acts as tumor suppressors or oncogenes [283, 284]. For instance, miR888 promotes the spread of the cancer to the body via metastasis and invasion [285]. miR-31, miR-34, miR-182, miR-211 and miR-599 expression are positively correlated with SATB2 in carcinogenesis [286]. miR-206 reduces PAX3 and MET gene expression and leading to the reduction the malignancy of osteosarcoma [287]. On the other hand, miR-181c and miR-133b have the opposite effect by increasing the sensitivity of chemotherapies [288]. However some studies exhibit miR133b cause resistance to chemotherapy [289]. miR-148a, miR-374b and miR-433 decrease cell proliferation and invasion in the pancreas, cervical and non-small cell lung cancer, respectively [290-292]. The outlined functions of miRNAs are new evidences to be targeted for chemotherapy [290].

Long non-coding RNAs, which are more than 200 nucleotides in length, act as negative or positive regulators in differentiation and development processes and provide homeostasis [293-

295]. Long non-coding RNAs are involved in epigenetic regulation, post-transcriptional regulation, could act as either a tumor suppressor or oncogene in cancer [296]. In addition, long non-coding RNAs maybe used for the purpose of early diagnosis and prognosis [274, 275]. lncRNA CASC11, lncRNA TUG1, lncRNA PCAT6, lncRNA LOC730100 and lncRNA LINK-A have important functions in the proliferation, metastasis, bladder cancer, laryngocarcinoma, cervical, glioblastoma and ovarian carcinoma [297-301]. Surprisingly, it was also observed that lncRNA MALAT1 had effects on energy metabolism that are known to be associated with metastasis in hepatocellular carcinoma and upregulation of glycolysis genes [302]. Thus, the relationship of lncRNAs involved in the Warburg effect, which is an important phenomenon in cancer metabolism, have been shown [302].

When mouse embryonic fibroblast cells were transfected with shPBX, expression of MEIS1 and MEIS1-PREP1a were also reduced at the protein levels [8]. MLL-AF4 siRNA treatment also led to reduction of the expression levels of HOXA9 and MEIS1 signaling pathway, which in turn, prevented engrafment and clonecity [104]. Besides, HoxA9 siRNA transfected THP cells demonstrated an increased apoptosis within 5 days [303]. When Human enhancer of zeste 2 (EZH2), a member of the family of polycomb repressive complex (PRC), is suppressed by siRNA, the expression levels of MEIS1 and HOXA9 are reduced in human acute leukemia cells [304]. In the pancreatic cancer cells (i.e. Panc1), mitochondrial activity was decreased by the suppression of TALE protein family members by siRNA [176]. Targeting of MEIS and its partners by siRNA suggest an avaiting potential for an *in vitro* chemotherapeutic approach by modulating related mitochondrial and metabolic pathways.

1.4. TARGETING MEIS, ITS ASSOCIATED PARTNERS AND PATHWAYS IN CANCER

Small molecules by binding to a macromolecule alter the activity or function of the target macromolecules [305]. Small molecules could inhibit protein-protein/DNA-protein interactions; therefore utilization of small molecules allows understanding of the biological processes and could be used in the treatment of various diseases [306]. Small molecules that are effective in cellular signalling pathways are easily diffused intracellularly thus can be

transported very rapidly inside of the intercellular region [307]. Except for protein based drugs, the majority of pharmaceutical active compounds are small molecules [308].

In the last decades, small molecules have been largely studied for their utilization in cancer therapy. MEIS1 has been shown to transactivate HIF-1α and HIF-2α expression, modulate cellular metabolism, and redox state [21, 309-311]. There is a direct correlation between HIF-1α and mitochondria activity in healthy cells. HIF-1α and HIF-2α protect the cell against ROS dependent apoptosis. Some of the small molecules that disrupt mitochondria activity via inhibiting HIFs lead to cancer cell apoptosis [312-314].

MEIS, associated HOX proteins and downstream pathways maybe prominent chemotherapy targets for cancer treatments. To this end, various studies in the last decade targeted the TALE family proteins and their interaction with MEIS proteins with small molecules or peptites in cancer. A peptide, for instance, designed to disrupt the HOX/PBX/DNA interaction reduced the proliferation of T3M4 pancreatic cancer cells, K562 leukemia cells and MJT1 melanoma cells [122]. Two small peptites HXR9 and CXR9 have been shown to induce apoptosis in non-small cell lung cancer cells, breast, ovarian, prostate and meningioma cells by distrupting the interaction of HOX and PBX [109, 315-317]. In neuroblastoma, stable PBX1-MEIS1 interaction provides overexpression of PHOX2B [76]. PHOX2B expression was reduced in neuroblastoma cells as a result of small molecule combination that includes curcumin, SAHA and trichostatin [76].

Several of the MLL mixed lineage leukemia cell lines have overexpressed MEIS1 and BCL2 [17]. As a result of small molecular library screening, it has been observed that CCI-007 small molecule, although it is not specific for MEIS1, has a cytotoxic effect within few hours in the CALM-AF10, MLL-r, and SET-NUP214 leukemia cell lines [17]. The small molecule CCI-007 triggers caspase-dependent apoptosis due to mitochondria depolarization and alters the expression of MLL-related genes [17]. MLL cell lines have different metabolic phenotypes and mitochondrial respiration through different molecular pathways [318]. CCI-006 also impairs mitochondria in the case of CCI-007 and increases apoptosis. Intriguingly, small molecule CCI-006 demonstrates a cytotoxic effect in MLL specifically in low MEIS expressing subset of cells. This suggests that MEIS+ cells may escape from CCI-007 induced apoptosis.

1.4.1. Novel Small Molecule MEIS Inhibitors

Studies in the last decade have shown that MEIS and its partner proteins have crucial roles in regeneration, stem cell/progenitor function, cellular metabolism, RLS and tumorigenesis [98, 319, 320]. Various cancers overexpress MEIS proteins and its cofactors.MEIS proteins have been found to interact with PBX1 and HOXA9 while driving tumorigenesis [100]. Small molecule inhibitors blocking PBX1-DNA interaction have been recently described [321]. These inhibitors disrupt DNA-protein interaction instead of protein-protein interaction, and therefore they are more efficient than previously developed inhibitors in terms of *in vitro* usage, cellular penetration, and/or solubility [321].

We have recently used our proprietary tools and expertise in MEIS biology to develop MEIS inhibitors [322, 323]. We have performed in silico, *in vitro*, *ex vivo* and *in vivo* assays to validate small molecule MEIS inhibitors. Newly developed small molecule MEIS inhibitors are cell-permeant and dose dependent. Newly identified two small molecule MEIS inhibitors (namely MEISi-1 and MEISi-2) are cell-permeable and dose dependent that specifically inhibits MEIS Homeodomain (HD)-target DNA interaction, thereby preventing the transactivation of MEIS targeted gene expression (**Figure 1.2.**). They have a high affinity and preferential binding to MEIS HD in comparison to other TALE family of hemeodomain proteins. They Inhibit MEIS-Luc activity in a dose dependent manner as low as 100 nM. Meis1 is known to transcriptionally regulate expression of Hif-1α, Hif-2α, and p21 [12, 21]. MEIS inhibitors downregulate the expression of Meis1, Meis2, and MEIS target genes including Hif-1α, Hif-2α, and p21. They demonstrate tissue and cell type specific phenotype, that MEISi-1 and MEISi-2 could induce human and mouse hematopoietic stem cell expansion, self-renewal and increased CFU-GEMM. They downregulate the expression of Meis1 and Hif-2α in the bone marrow. They induce mouse hematopoietic stem cell expansion *in vivo*.

Studies conducted over the last decades suggest that MEIS and MEIS partners are crucial for cancer biology. The development of small molecules targeting MEIS in drug development can be a valuable asset in chemotherapy of MEIS positive cancers.

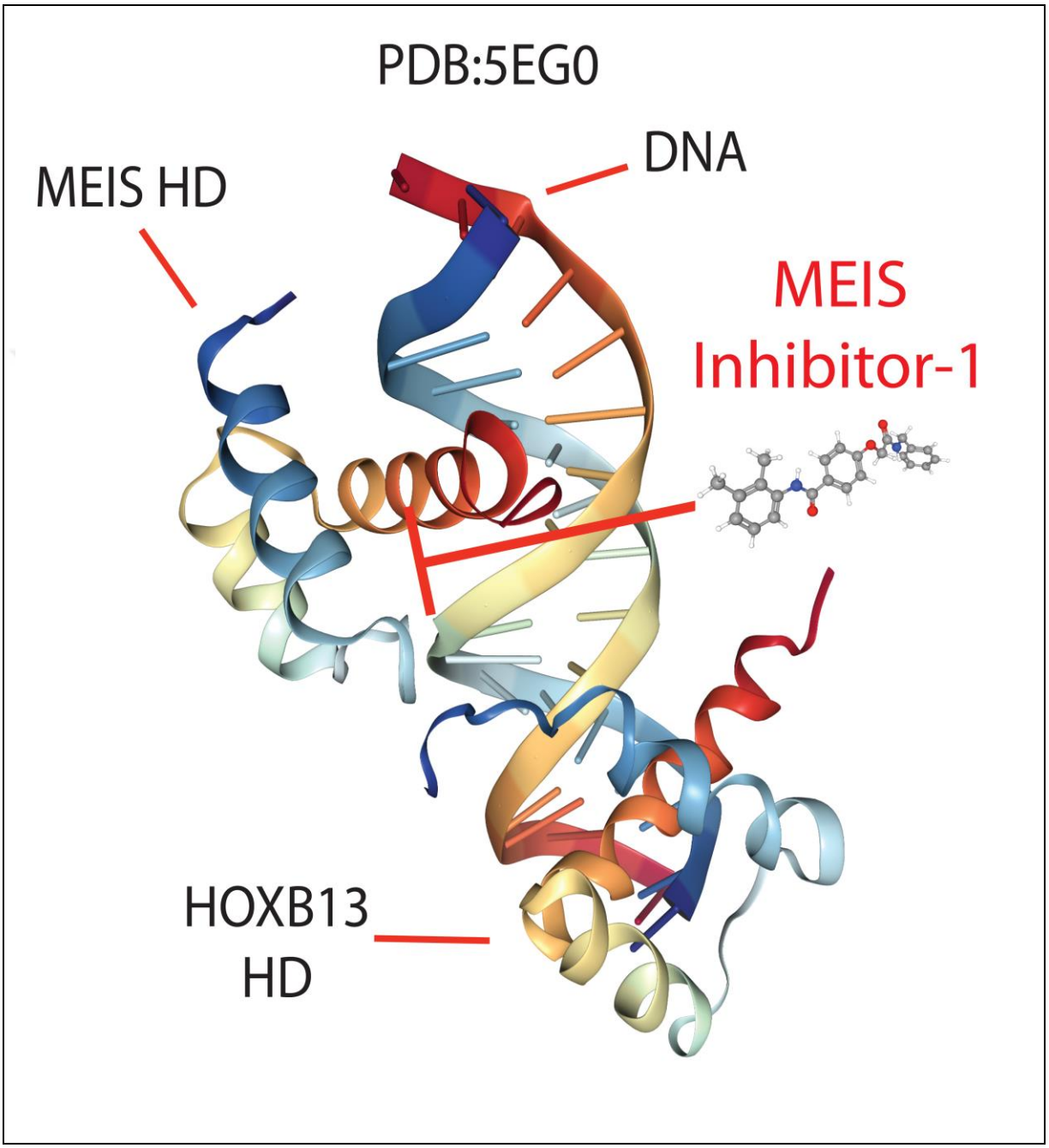


Figure 1.2. Newly developed small molecule MEIS inhibitors targets HD-DNA interaction. Crystal structure of MEIS and HOXB13 homeodomains bound to DNA have been shown.

2. MATERIALS AND METHOD

2.1. CELL CULTURE

PCa human cancer cell lines were cultured in RPMI 1640 (Gibco, Cat no: 11875101) completed with 10 percent FBS (Gibco, Cat. No: 10270-106) and one percent PSA (Antibiotic/Antimycotic Solution, HyClone, Cat. No: SV30079.01) at 37^{o}C having five percent CO_2.

RPMI media containing 10 percent FBS and one percent PSA was warmed in 37 ^{o}C before use. 9 mL of warm total media was placed into 15 mL rotator tube (Falcon, Cat. No: 352096). 1 cryovial containig cell line of PCa cell lines was taken from - 80 ^{o}C fridge and defrosted, and then dissolved with one ml completed RPMI media. Thawed cell suspension was added on media that was placed into 15 mL rotator tube gradually. Tubes were centrifuged at 1500 RPM for five min at RT. Subsequent to disposing of supernatant, pellet was resuspended in three to five mL of warm total media and moved into T25 flask.

For passaging, the medium was refreshed within two to three days until the cells reached to 70 percent density and then the flask was washed with PBS and collected by trypsinization. The cell-trypsin solution, which was inactivated with the completed medium as much as the amount of trypsin, was precipitated at 1500 rpm for five min. The cell pellet was resuspended with one ml of fresh medium and finally moved into new flasks at the appropriate density.

Untill cell passage, all of sub-process were applied and then cell were counted. About $1x10^{6}$ cells calculated for freze. Cryovials (IsoLab, Cat. No: I.091.11.102.100) were labelled and the inoculum was centrifuged at 1500 RPM for five min at RT. Supernatant was discarded and the pellet was dissolved in one ml freezing buffer (10 percent DMSO in FBS). Cells were moved to -80 °C fridge.

2.2. CHEMICALS

MEISi-2 inhibitor with a molecular weight of 306.21 g was prepared 10 mM main stock in DMSO as a solvent. Afterwards, intermediate stocks were prepared and stored in 10 different concentrations using the serial dilution method.

2.3. CELL VIABILITY ASSAY AND IC50

PCa cell lines were seeded at appropriate densities in a 96-well plate on day zero(Costar, Cat. No: 3596). The medium in the 96-well plate was refreshed to remove dead cells in culture on day one and then MEISi-2 treatment interested concentration also it used DMSO way used a control vehicle at indicated was done.

On day three, 100 µL CellTiter 96 Aqueous One Solution Cell Proliferation Assay (Promega, Cat. No:G3581) was applied to cells in 20 percent in FBS containing RPMI. In addition some wells were used for blank subtraction. After two h incubation in 37 º C incubator, absorbances were measured at 490 nm via microplate reader (Thermo Scientific Varioskan Lux). IC50 and analysis of cell viability was determined by using IC50 Calculator | AAT Bioquest and Graphpad Prisms 8, respectively.

For real cell count, all of process were similar to cell viability assay. Instead of Aqueous One Solution, 10 uM Hoescht 33342 (Thermo Scientific Fischer, cat no: 62249) was applied in order to dye cell nuclei. After 15 min incubation at cell incubator, cells were moved to automated fluorescence Cytell Cell Imaging System (Image Solutions). Data was analyzed via Graphpad Prisms 8.

2.4. PROGRAMMED CELL DEATH (APOPTOSIS) ANALYSIS

Trypsinated de-attachment adherent cells were obtained by centrifugation and treated to Annexin V and propidium iodine in one and two percent in PBS according to the manufacturer's recommendation. (Invitrogen, cat no: BMS500FI-20) and data was recorded in CytoFlex Flow Cytometer (Beckman Coulter).CytoFlex Flow Cytometer software is used for analysing of data (Beckman Coulter) in order to determine apoptotic population distributions.

2.5. mRNA EXPRESSION ANALYSIS USING REAL-TIME PCR

Total RNA was isolated from MEISi-2 treated cells using one phase RNA purification, Nucleozol, (Machereyy- Nagel, cat no: 740404.200) following to the manufacturer's recommendations. NanoDrop 2000 was used to measure RNA concenration and to arrange the RNA concentratin. 0.5 to two μg mRNA was used as a template RNA in order to reverse transcribe in a reaction mix containing one μL Oligo (dT)18 primer (Thermo scientific fischer), four uL 5X Reaction Buffer (Thermo scientific fischer), one uL RiboLock RNase Inhibitor (20 U/μL) (Thermo scientific fischer), two uL 10 mM dNTP Mix (Thermo scientific fischer) and two μL RevertAid M-MuLV RT (200 U/μL (Thermo scientific fischer) and complete 20 μL with nuclease free water for one h at 42°C and five min at 70°C followed thank to dilution with nuclease free water 10 ng/μL cDNA was obtained.

Quantitative real-time RT-PCR (qRT-PCR) reactions contained 20 ng cDNA, 2x Applied Biosystems™ PowerUp™ SYBR™ Green Master Mix (Thermo Fischer Scientific, cat no: A25743), one uL interest forward and reverse primers (100 μM) for total 10 μL reaction/well. The all of primers were ordered from Sentegen (Turkey). All samples was performed in duplicate with a LightCycler 96 (Roche). Data analysis was performed using ΔΔCT method by Graphpad Prisms software. Compared to the DMSO control group, the data were analyzed by normalization using the ΔΔCT method.

Table 2.1. Forward and reverse primers used in Real Time PCR

Gene (human)	**Forwardprimer**	**Reverseprimer**
Actin	AGGCACCAGGGCGTGAT	GCCCACATAGGAATCCTTCTGAC
Meis1	GGGCATGGATGGAGTAGGC	GGGTACTGATGCGAGTGCAG
Meis2	GAAAAGGTCCACGAACTGTGC	CTTTCATCAATGACGAGGTCGAT
Meis3	ATGGCCCGGAGGTATGATGA	GAAGAGCGGGTGTCCATAGA

Pbx1	CATGCTGTTAGCGGAAGGC	CTCCACTGAGTTGTCTGAACC
Pbx2	ATGGACGAACGGCTACTGG	CCCCGATGTCTTGCTTCCC
Pbx3	ATTACAGAGCCAAATTGACCCAG	TCTCGGAGAAGGTTCATCACAT
Pbx4	TCCGTGGCATTCAAGACGAAG	TGGTAAATCTGTCGGATCTGGG
Hif1A	TTCCCGACTAGGCCCATTC	CAGGTATTCAAGGTCCCATTTCA
Hif2A	CGGAGGTGTTCTATGAGCTGG	AGCTTGTGTGTTCGCAGGAA
Hif3A	GCACCCTCAACCTCAAGGC	GCAATCCTGTCGTCACAGTAG

2.6. DETERMINATION OF PROTEIN EXPRESSION

MEISi-2 treated cell pellets were dissolved using 100 μL 1X RIPA Buffer for lysis (Thermo Scientific Fischer, cat no 10017003). Protein concentration of unknown samples were determined using a stardard curve prepared by bicinchoninic acid (BCA) assay kit (Takara, cat no T9300A). 25 to 50 ug protein was denatured, loaded into prepared 10 per cent SDS gel and run. Proteins were moved to polyvinylidene difluoride membranes (PVDF), 0,45 uM (Thermo Fischer Scientific, cat no 88518) and membranes were blocked with five per cent non-fat dairy milk (NFDM) or five percent bovine serum albumin (BSA) in Tris-Buffered Saline (TBS) containing 0.05per cent Tween-20 (TBS-T) for one h before incubation with primary antibodies (mouse anti-MEIS1-2-3 (1:200, Santa Cruz, cat no: sc-101850), Mouse-anti B-actin 1:1000 Santa Cruz, cat no sc-47778). Primary antibodies were diluted in TBS-T five per cent NFDM or five percent BSA and stay with membranes during all night at four°C. TBS-T were poured on membranes for washing and then membranes were incubated with secondary anti-mouse polyclonal (1:10,000, proteintech cat 10283-1-AP) in TBS-T five per cent NFDM or five per cent BSA for one h at 25 °C. After TBS-T steps, SignalFire™ Plus ECL Reagent was treated (Santa Cruz, cat no 12630) for one minute and chemiluminescence was detected using ChemiDoc XRS+(Biorad). For different protein determination on same membrane, using striping for reprobing buffer was used previous membranes (Abcam)for five- 10 min at room temperature with shaking, PBS was used as washing agent. Washing with TBS-T, then it was

ready for re-blocking in TBS-T with five percent NFDM or five percent BSA for one h at 20-25 °C. Levels of protein expression were determined via ChemiDoc XRS+ software.

2.7. ANALYSIS OF INTRACELLULAR MEIS1-2-3 EXPRESSION BY CYTOFLEX FLOW CYTOMETER

After trypsin, single cell suspension were fixed fixed with three- four per cent paraformaldehyte diluted in PBS (10 µg/ml) for 15-20 min in water bath. After washing, Triton-X, permebilization agent was used as 0.1 percent in same solvent for 10 min at 37°C. For antibody incubation, MEIS1-2-3 (santa cruz, sc-101850) diluted and incubated in five per cent BSA in a dark. Cells were carried to 95-well plate and samples were perform on a CytoFlex Flow Cytometer (Becham Coulter). Data were analyzed by CytoFlex Flow cytometer softwate (Beckham Coulter) to calculate mean fluoresence intensity of MEIS1-2-3.

2.8. MEASUREMENT CELLULAR ROS BY DCFDA

ROS was assayed using 2',7'-dichlorofluorescin diacetate (DCFDA) cellular ROS detection assay kit (Abcam # ab113851) following manufacturers' protocol. For ROS measurement, cells seeded appropriate density in six-well plates (200,000 cells per well) at D0. Cells were harvested by tripsinization and then incubated in 20 µM DCFDA in media without FBS for 30 min 37°C. Samples were performed and analyzed using the CytoFlex Flow Cytometer (Beckham Coulter) and its software, respectively.

2.9. CELL CYCLE ANALYSIS

MEISi-2 treated cells in suspension were incubated in Hoescht 33342 (10 ug-ml) for 45-60 min at cell incubator. After washing, Propidium Iodide (one µL / 100 µL, 20 µg/mL, invitrogen) diluted in PBS was used to determine dead cells. Samples were kept in a closed box to protect from light and incubated for 15 min before carried to 96- well plate. CytoFlex Flow Cytometer (Beckham Coulter) was used for analysis.

2.10. SCRATCH ASSAY

The metastatic ability of Pca cells was questioned by the scratch test. At D0, cells were seeded with as high density as possible in a 6-well plate. In D1, cells were treated with MEISi-2 and

subsequently scratched with a 200 μL sterile pipette tip. Photographs of the wells were taken at 0h and 48h with the aid of an invert microscope and the scratch in D0 was accepted as 100 percent and then quantitated by the ImageJ software (V. 1.47; NIH, Bethesda, MD, USA). The data were calculated as the percentage of PCa migration by the relation of closing the scratch.

2.11. STATISTICAL ANALYSIS

Statistical analysis was done with the GraphPad Prism 8.0.1 Software. Statistical differences were calculated with student-t test and one way anova: *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$; ****:<0.0001.

3. RESULTS

3.1. PCA CELL LINES EXPRESS MEIS1-2-3 MRNA AND PROTEIN

RT-PCR and Western Blot experiments were performed to check if MEIS1-2-3 expression was present in PCa cell lines. As a result of these studies, it was determined that MEIS1-2-3 mRNA and protein expression were in the relevant cells.

In the Meis1-2-3 mRNA expression comparison, PC-3 and DU-145 cells were shown to have a higher expression level, respectively. 22Rv-1 and LNCAP are found to express significantly less gene level mRNA Meis1-2-3 (Figure 3.1.a). PC-3 and DU145 expressed almost same MEIS1/2/3 and then 22Rv-1 and LNCaP's MEIS expression were 10- fold lower than PC-3 and DU145 (Figure 3.1.a) On the other hand, in terms of PBX1-4 had similar trend in PCa cell lines. PBX expression of PC-3 and DU145 were almost three in result of ACTB normalisation. On the contrary, 22Rv-1 and LNCaP's PBX expression were 10-fold lower than PC-3 and 22Rv-1 except PBX3 of 22Rv-1.(Figure 3.1.a)

A completely opposite trend is observed in MEIS1-2-3 protein expression in comparison to mRNA expression in PCa cell lines. LNCAP has the highest protein expression while PC-3 is the least. In fact, mRNA and protein expression are fully negatively correlated (Figure 3.1.b).According to normalisated protein levels of Pca cell lines, LNCaP' MEIS protein expression was 50 and it was the highest expression in Pca cell lines. After that MEIS protein expression of 22Rv-1, DU145 and PC3 followed LNCaP, respectively (30, 20, 5 calculated by MEIS1-2-3/ ACTB*100) (Figure 3.1.b). Statistical analysis were not done because Pca cells were classified in regard of MEIS expression.

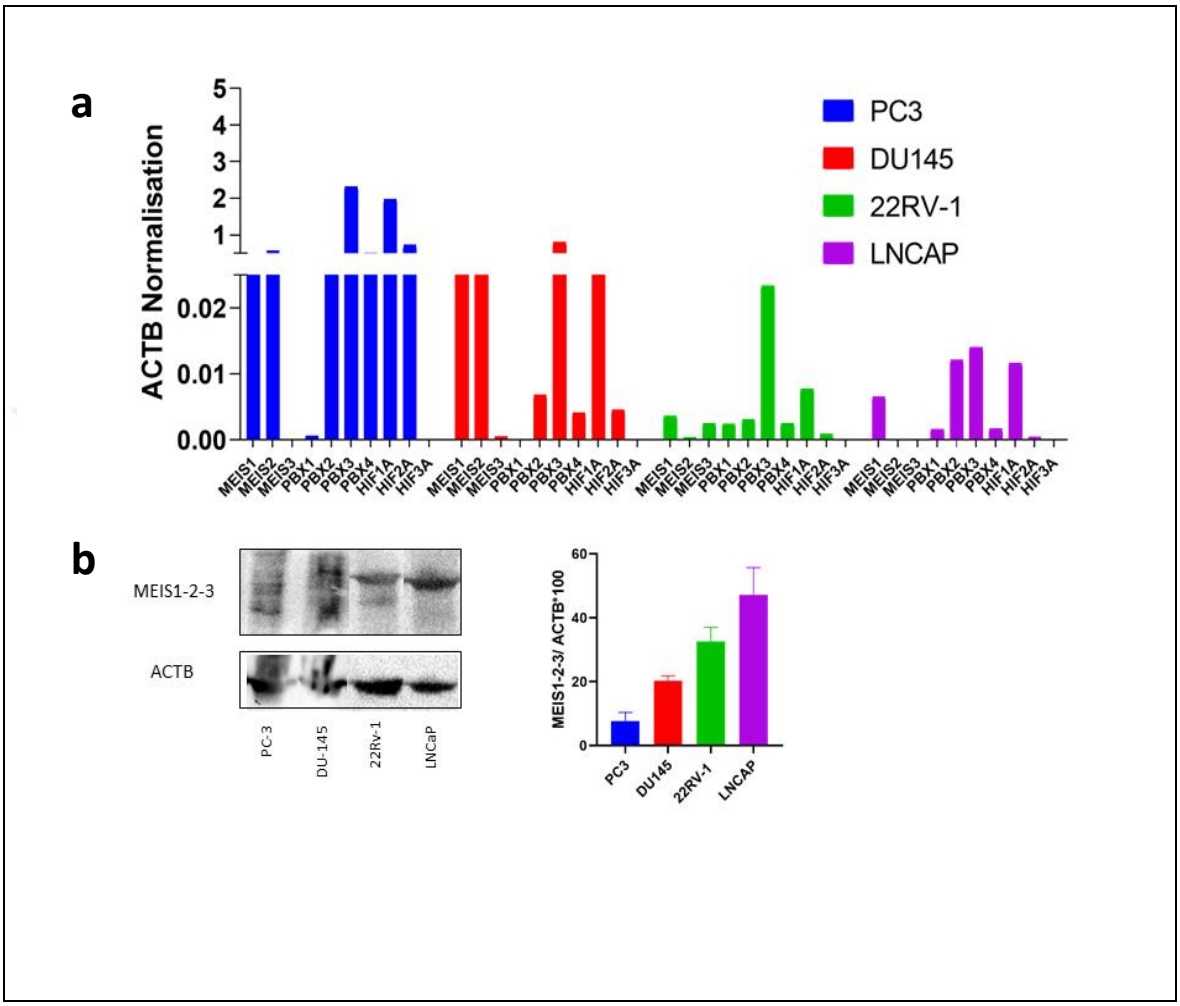


Figure 3.1. PCa cell lines express MEIS1-2-3 mRNA and protein. Total mRNAs of untreated PCa cells were converted into cDNA and then RT-PCR experiment was performed with them (a). MEIS1-2-3 protein expression of untreated PCa cells (b). Pca cells were ordered in regard of MEIS expression. For this reason statistical analysis were not done.

3.2. IC50 VALUES OF MEISi-2 IN PCA CELL LINES

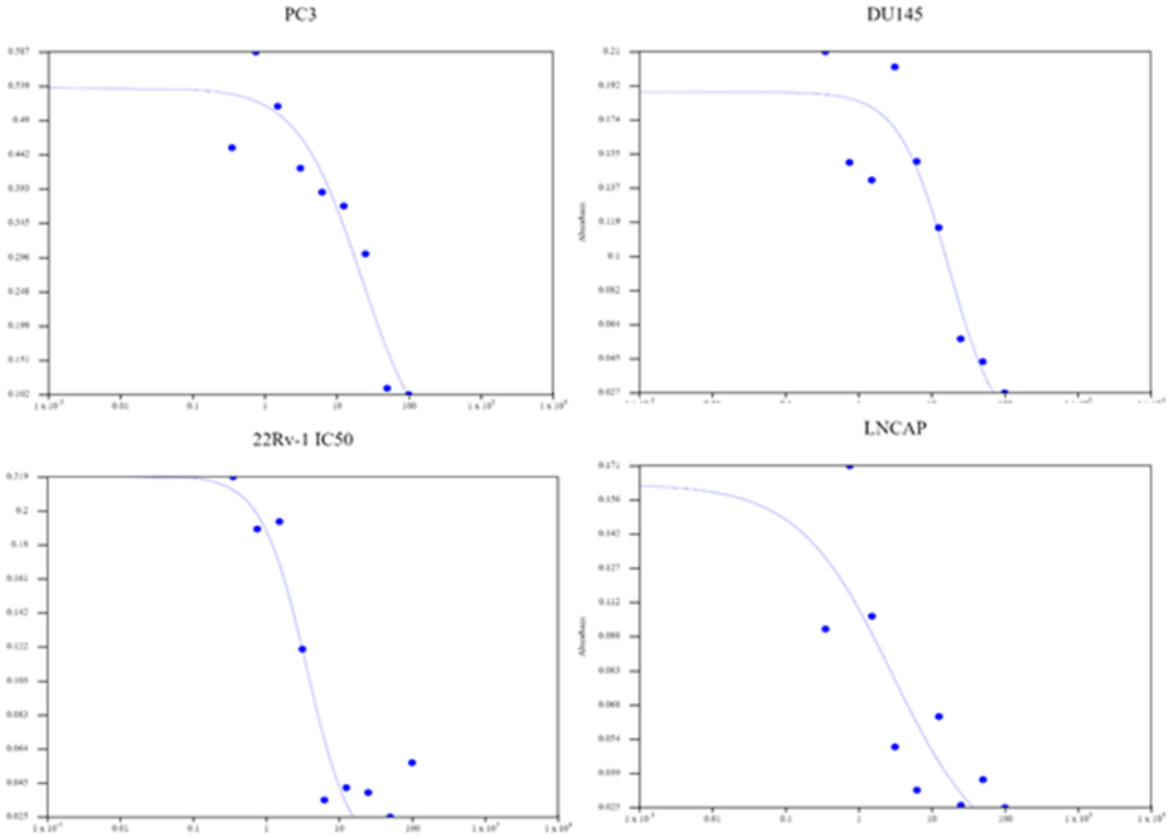
The toxic effect of MEISi-2 on PCa cell lines was investigated. In the MTS-based experiment, it was shown that the MEISi-2 treatment caused a pronounced toxic effect in PCa cell lines

(Figure 3.2.). It clearly shows that there is an inverse relationship between MEIS1-2-3 protein expression and IC50 values(Figure 3.2. ,a and b). This expected situation may be that MEISi-2 binds to the MEIS homeodomain, preventing it from binding to DNA. PC-3, DU145, 22Rv-1 and LNCaP's IC50 values were 21, 95 uM, 17,96 uM, 3,18 uM and 2,87 uM, respectively (Figure 3.2.,b).

In addition, treatment of five μM, 10 μM, and 20 μM MEISi-2 resulted in a significantly reduced cell viability in all PCa cell lines relative to control DMSO at the 48 h (Figure 3.2.c). When comparing all PCa cell lines, a similar relationship to protein level-IC50 value was determined in cell viability. This finding suggests that MEISi-2 is a potential chemotherapy agent for PCa.

In addition, in order to validate cell viability experiments, Hoescht stained cells as a result of MEISi-2 treatment were photographed with automatic fluorescence microscopy and then the data were analyzed. Hoescht dye are able to bind DNA of live and dead cellls. Except for the 22Rv-1 cell line, the results were quite similar (Figure 3.3.). In 22Rv-1, however, most likely dead cells increased the cell number as they fixed on the surface of the 96-well plate (Figure 3.3. c). Only in the experiment with cell counting the significance appears to have dropped slightly. The main reason for this may be the same cells fixed at 22Rv-1 as described above.

a



b

CELL	IC50 Value
PC-3	21, 95 uM
DU-145	17,16 uM
22Rv1	3,18 uM
LNCAP	2,87 uM

c

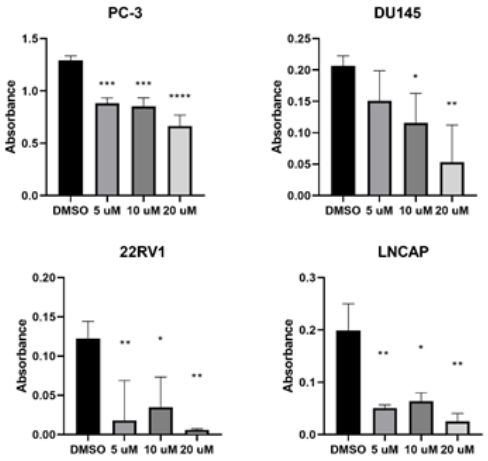


Figure 3.2. MEISi-2 treatment causes toxic effects on PCa cells. PCa cells show sensitivity to the 2-day MEISi-2 treatment compared to the DMSO control group. IC50 values of PCa cells were calculated (a,b). Treatment of 5 μM, 10 μM and 20 μM MEISi-2 sharply reduces cell viability (c). Student t-test used in order to determine P- values represented in the figure (n=3, *p<0.05, **p<0.01, ***p<0.001,****p<0,0001)

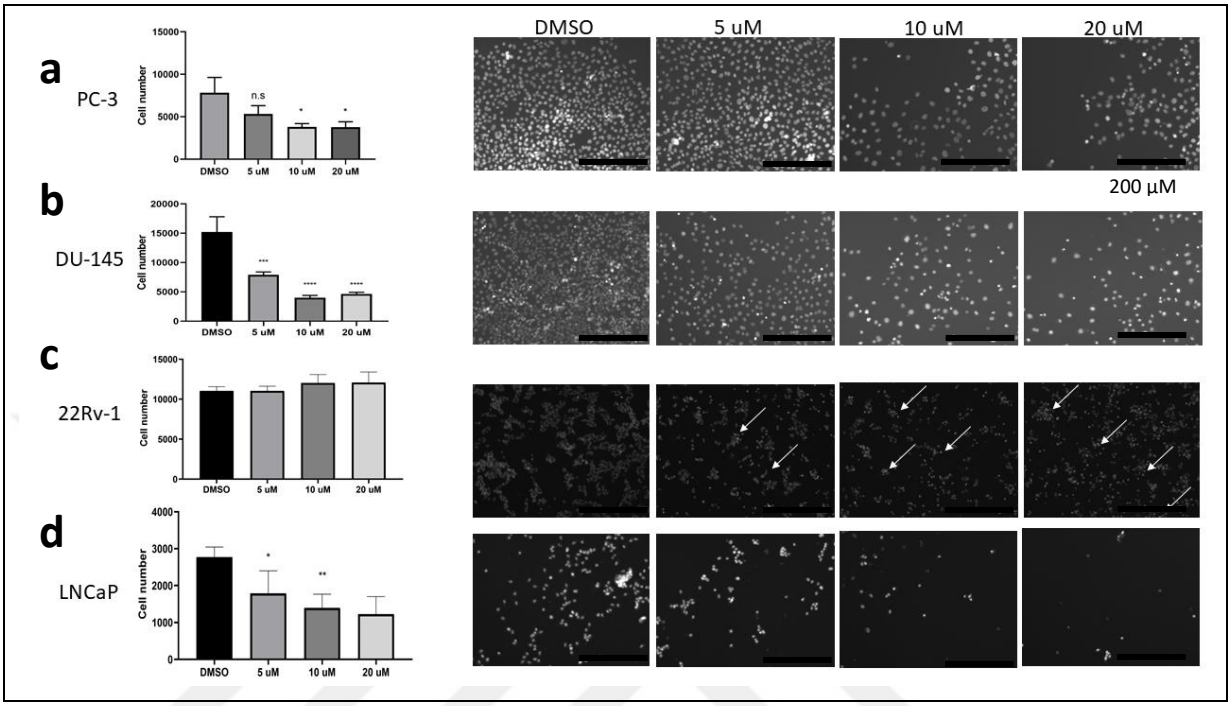


Figure 3.3. Real cell count was determined by automatic fluoresence microscopy via using Hoescht stained Pca cells. (a) PC-3, (b) DU-145, (c) 22Rv-1 and (d) LNCAP indicate the change of real cell count due to MEISi-2 treatment for 48 hours and representative images were shown real cell count of Pca cell lines. White arrows indicate fixed dead cells. All scale bars are 200 µM. Student t-test used in order to determine P- values represented in the figure (n=3, *p<0.05, **p<0.01, ***p<0.001, n.s: non-significant)

3.3. MEISi-2 TREATMENT INCREASES APOPTOSIS IN PCA CELLS

The level of apoptosis was examined as it may be the reason for the decrease in cell viability as a result of MEISi-2 treatment. Treatment with five μM, 10 μM and 20 μM MEISi-2 demonstrated a significant increase in apoptosis in PCa cells, except for PC-3. (Figure 3.4.) MEISi-2 treatment in PC-3 dose-dependently increased total apoptosis, even 20 μM MEISi-2 treatment increased total apoptosis almost two-fold but not statiscally significant (Figure 3.4., b and c). On the other hand, the dose-dependent effect of MEISi-2 treatment in PC-3 and DU-145 cells was very obvious (Figure 3.4, b-d). Interestingly, five μM MEISi-2 treatment was the most effective in 22Rv-1 cell (Figure 3.4,f and g). Finally, although LNCaP was the cell mosts to the sensitive MEISi-2 treatment, it did not cause a significant apoptosis increase with the low dose (1,25 uM and 2,5 uM) MEISi-2 treatment (Figure 3.4,h and ı).

When closer look early apoptosis(DAPI negative and Annexin V positive staining), PC-3's early late apoptosis increased dose- dependent post MEISi-2 for 48h. 5 μM MEISi-2 resulted in almost 3 percent early apoptosis although 20 μM MEISi-2 led to 40 percent early apoptosis. Dose-dependent trend of MEISi-2 were similar to DU-145. While the lowest MEISi-2 caused just about 2.5 percent early apoptosis, the highest one were almost ten percent (Figure 3.4, a). On the other hand, in terms of 22Rv-1 5 μM MEISi-2 caused the highest early apoptosis proportion in among other doses (Figure 3.4, a). For LNCaP, there was no significant early late post MEISi-2 treatment.

Additionally, the proportion of late apoptosis for PC-3, DU-145, 22Rv-1 and LNCaP had a plateu in MEISi-2 treatment with different doses (Figure 3.4, a). Ultimately, MEIS inhibitor treatment was responsible for necrosis increase in regard of LNCaP (Figure 3.4, a and h). For 22Rv-1 and DU-145, MEIS inhibitor treatment caused a little bit necrosis increase in all doses (Figure 3.4, a, d and f). However, increasing the doses of MEIS inhibitor treatment increased necrosis proportion (Figure 3.4, a and b).

3.4. MEISi-2 TREATMENT INCREASES CELLULAR ROS

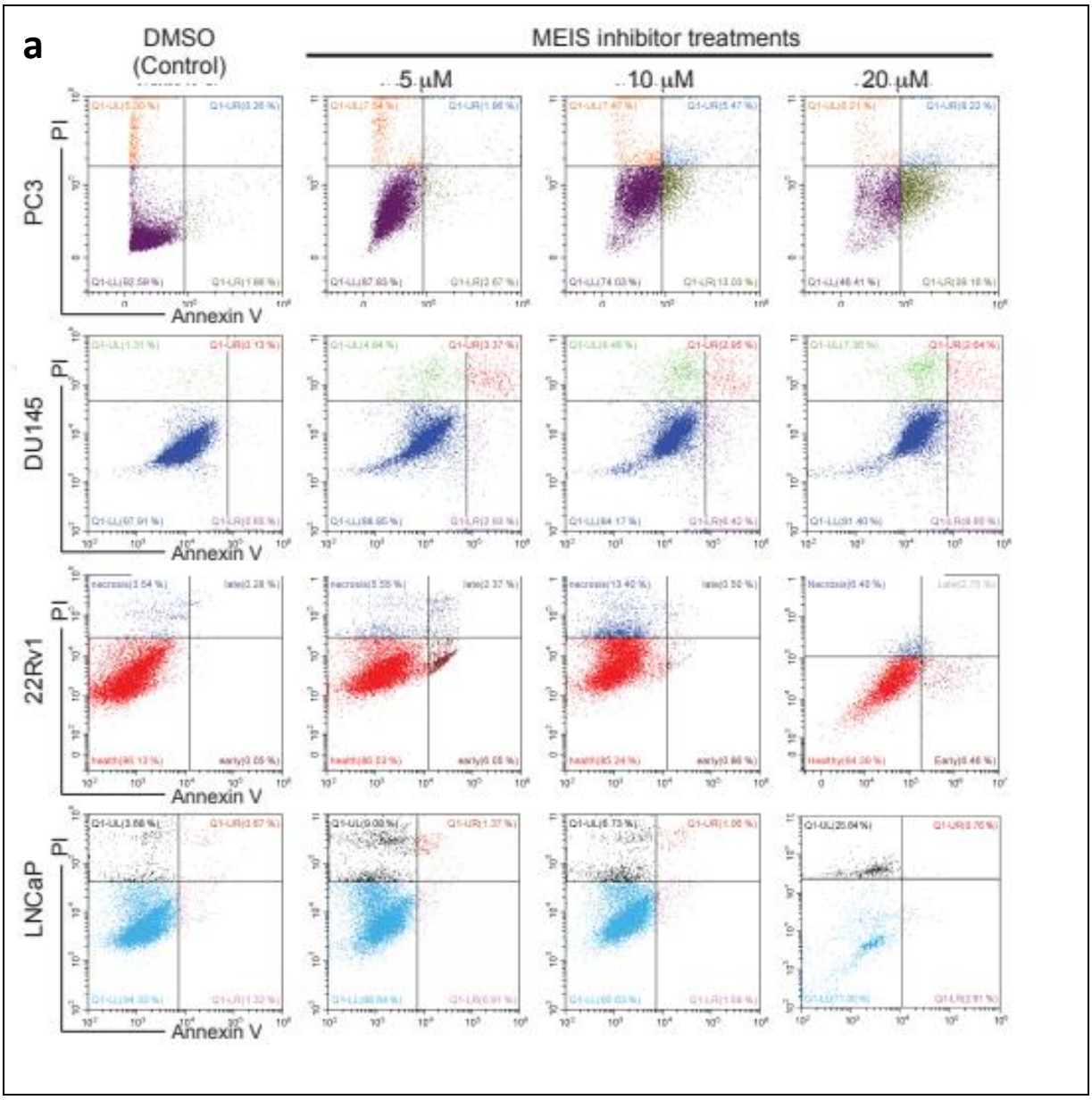
It is known that MEIS1-2 proteins regulate the expression of HIF1-3α genes by binding to the promoter regions. So how the MEISi-2 treatment altered the cellular ROS level was investigated next.

It seemed that DMSO group in PC-3 did not produce intracellular ROS. However, MEIS inhibitor dramatically increased intracellular ROS level. 5 μM MEIS inhibitor significantly inreased ROS level and 10 μM MEISi-2 treatment's ROS level was 2- fold higher than 5 μM.Despite being 10- fold higher than 10 μM, the 20 μM MEISi-2 revealed 0,057 p-value (Figure 3.5, a).

Surprisingly, the increase of MEIS inhibitor concentration and intracellular ROS levels of PCa cell lines were negatively correlated each other. 5 μM MEIS inhibitor caused almost 4-fold intracellular ROS level in DU-145. Although all concentration of MEIS inhibitor in DU-145 were significantly effective, 20 μM MEIS inhibitor treatment increased 2- fold ROS level than DMSO (Figure 3.5, b).

For 22Rv-1, 10 μM and DMSO had about same intracellular ROS levels after 48 hours. Additionally, 5 μM MEIS inhibitor effect were 25 percent higher than DMSO. Interestingly, the highest increase of ROS level seemed to 20 μM MEIS inhibitor but there was no significant effect statistically (Figure 3.5, c).

Finally, the dose-dependent of MEIS inhibitor for ROS levels of LNCaP was obvious.5 μM MEIS inhibitor effect was 2,5 fold higher than DMSO and higher doses of MEIS treatment (10 and 20 μM) were slighlty higher than 5 μM MEIS inhibitor effect (Figure 3.5, d).


a
DMSO
(Control)
MEIS inhibitor treatments
5 μM
10 μM
20 μM
PC3
DU145
22Rv1
LNCaP
PI
Annexin V

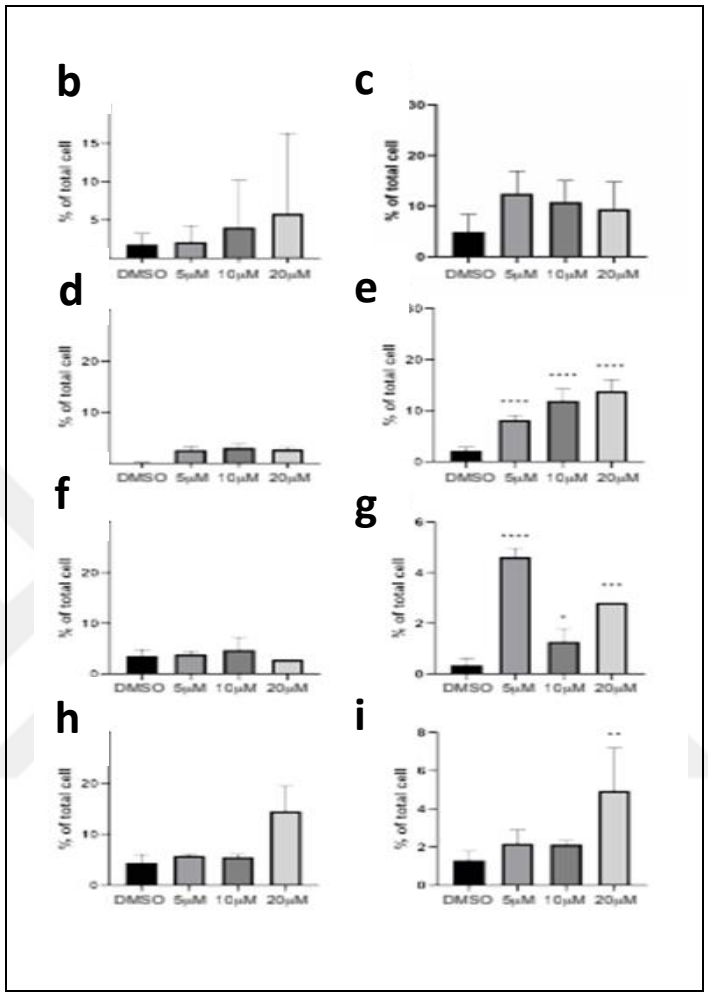
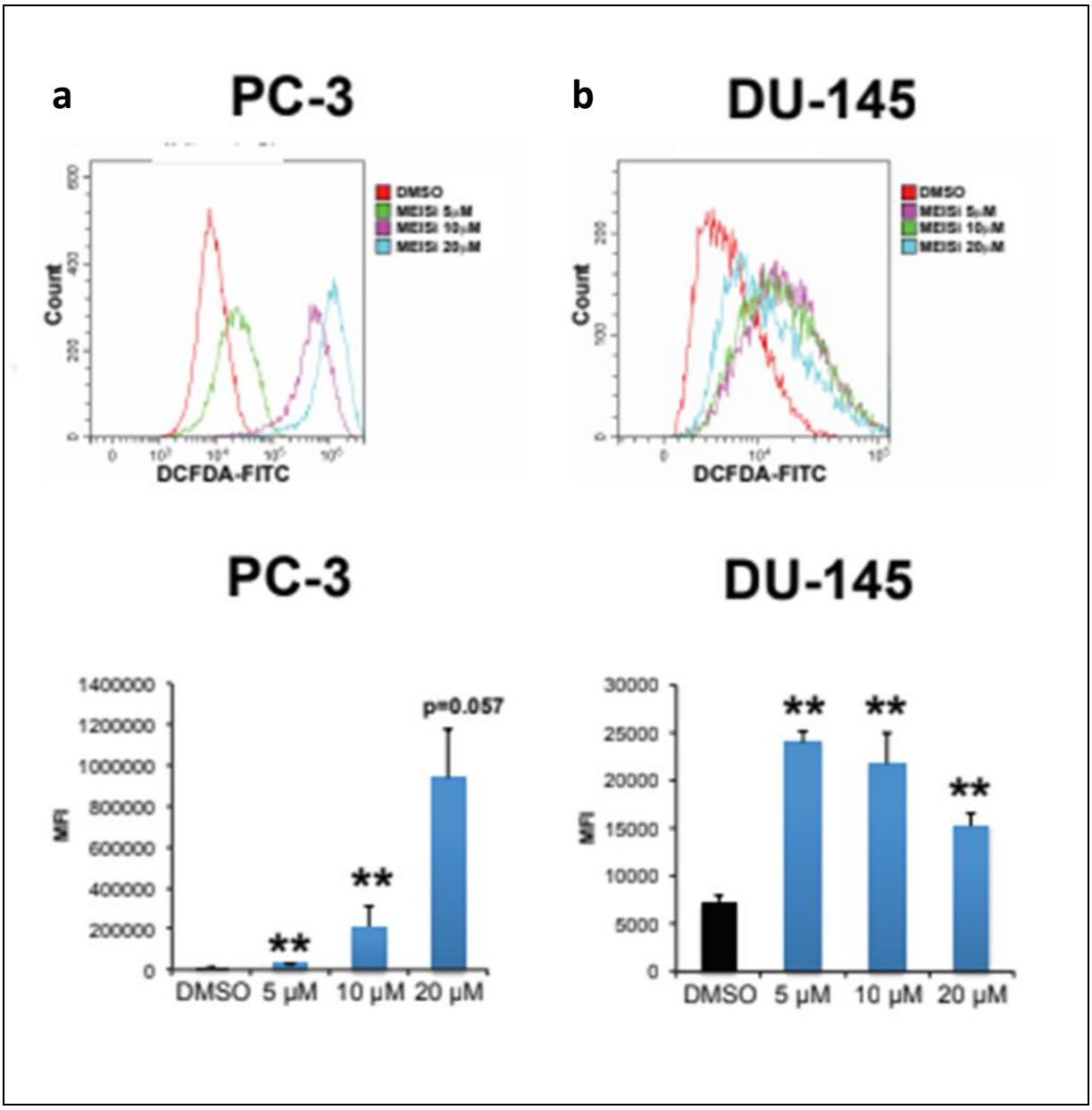


Figure 3.4. MEISi-2 treatment increases apoptosis in PCa cells for 48 hours. Total apoptosis was increased in (a) PC-3, (b) DU145, (c) 22Rv-1 and (d) LNCaP cells as a result of two-day MEISi-2 treatment. Bar graphs showed that the amount of necrosis and apoptosis compared to DMSO control for (b and c) PC-3, (d and e) DU-145, (f and g) 22Rv-1 and (h and i) LNCaP, respectively. Also shown in representative images. Student t-test used in order to determine P-values represented in the figure (n=3, *p<0.05, **p<0.01, ***p<0.001, n.s: non-significant)


a
PC-3
b
DU-145
Count
DCFDA-FITC
DMSO
MEISI 5μM
MEISI 10μM
MEISI 20μM
PC-3
DU-145
MFI
p=0.057
**
DMSO
5 μM
10 μM
20 μM

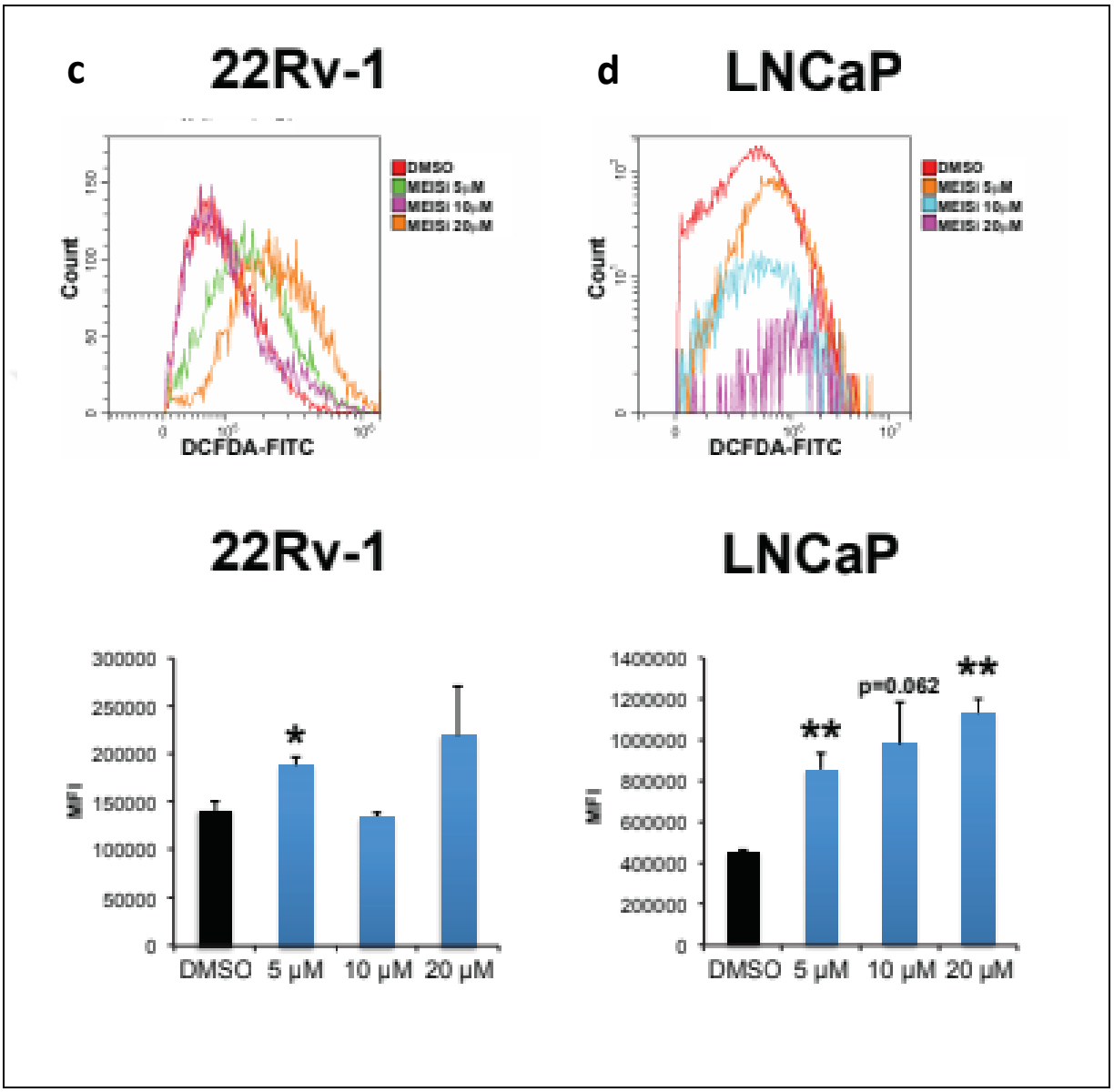


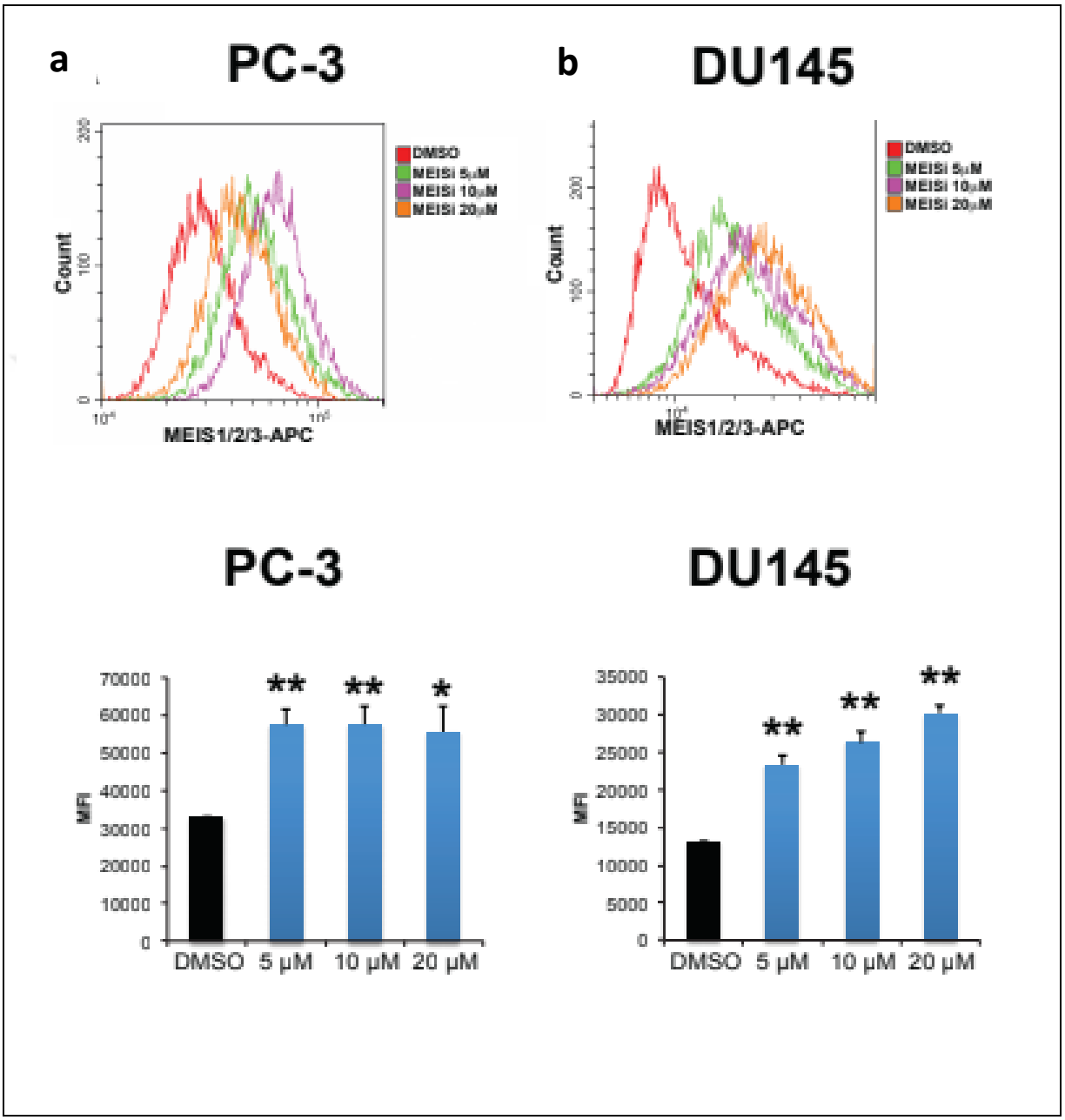
Figure 3.5. The effect of MEISi-2 treatment on the amount of intracellular ROS was investigated. The overlay histograms show the alterations of intraceullar ROS levels for (a) PC-3, (b) DU-145, (c) 22Rv-1 and (d) LNCaP at 48 hours. DCFDA: 2′,7′-Dichlorofluorescin diacetate. Student t-test used in order to determine P- values represented in the figure (n=3, *p<0.05, **p<0.01, ***p<0.001)

3.5. MEISi-2 TREATMENT ENHANCES INTRACELLULAR MEIS1-2-3 EXPRESSION

As a result of the MEISi-2 treatment, the amount of MEIS1-2-3 protein expression in the cells was examined. As a result of the two-day treatment of MEISi-2, it was determined by flow cytometry and Western Blot experiments that the amount of MEIS1-2-3 protein increased in all PCa cells (Figure 3.6.,3.7.).

Five, 10 and 20 μM MEIS inhibitor treatment increased 2- fold MEIS protein expression compared to DMSO. Despite same MEIS expression, the p-value of 20 μM MEISi-2 were lower than other MEIS inhibitor treated groups (Figure 3.6, a). On the other hand, the dose- dependent effect of MEIS inhibitor were realized at DU-145 and LNCaP cell lines (Figure 3.6, b and d). Five μM MEISi-2 treatment caused almost 1.5 fold MEIS1/2/3 expression in DU-145. Increasing MEIS inhibitor concentration, MEIS1/2/3 expression increased (Figure 3.6, b). In regard of LNCaP, 0,6 μM MEIS inhibitor treatment did not increase intracellular MEIS expression, despite 1,25 and 2,5 μM MEIS inhibitor had significant effect for intracellular MEIS expression (Figure 3.6, d). Compared to DMSO, 2,5 μM MEIS inhibitor did not alter intracellular MEIS expression in 22Rv-1, but then μM MEISi-2 signicantly increased intracellular MEIS expression (From 2500 M.F.I to 3000 M.F.I). Finally, this increase were reduced by 10 μM MEIS inhibitor treatment.

In addition, the flow cytometry results were confirmed by the increase in the MEIS1-2-3 protein level as a result of five μM MEISi-2 treatment by Western blot. Interestingly, the change in the MEIS1-2-3 protein level was not significant, as in flow cytometry (Figure 3.7.).


a
PC-3
b
DU145
Count
MEIS1/2/3-APC
DMSO
MEIS 5μM
MEIS 10μM
MEIS 20μM
PC-3
DU145
MFI
70000
60000
50000
40000
30000
20000
10000
0
35000
30000
25000
20000
15000
10000
5000
**
*
DMSO
5 μM
10 μM
20 μM

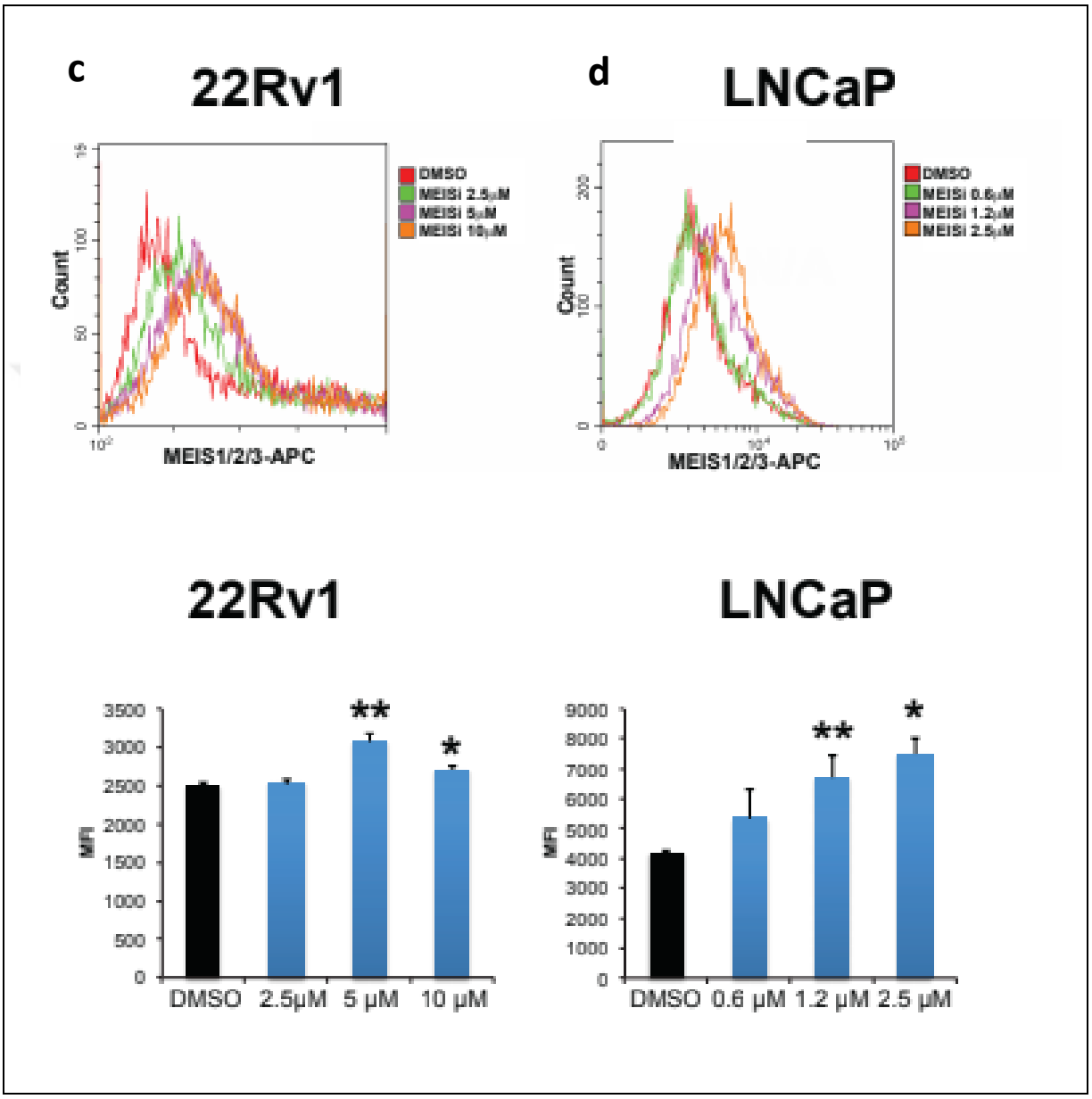


Figure 3.6. Intracellular MEIS1/2/3 expression were measured via using flow cytometry. (a) PC-3, (b) DU-145, (c) 22Rv-1 and (d) LNCaP's histogram overlays indicated the altereations of MEIS expression in prostate cancer cell lines. Student t-test used in order to determine P- values represented in the figure (n=3, *p<0.05, **p<0.01, ***p<0.001)

After five μM MEIS inhibitor treatment in PC-3, MEIS1/2/3 expression were higher than DMSO (almost 50 percent) at 48 hours (Figure 3.7, a). The gap between MEIS inhibitor treated and DMSO group were higher than the gap of PC-3. The increase MEIS expression of DU-145 was above two-fold than PC-3 (Figure 3.7, b). Also, the increase of MEIS expression in 22Rv-1 were just below 2- fold compared to DMSO (Figure 3.7, c). Surprisingly, the result of MEIS inhibitor treated LNCaP cells were really different other cell lines and flow cytometry. Ultimately, MEIS inhibitor treatment led to reduction of MEIS expression in LNCaP, 35 percent (Figure 3.7, d).

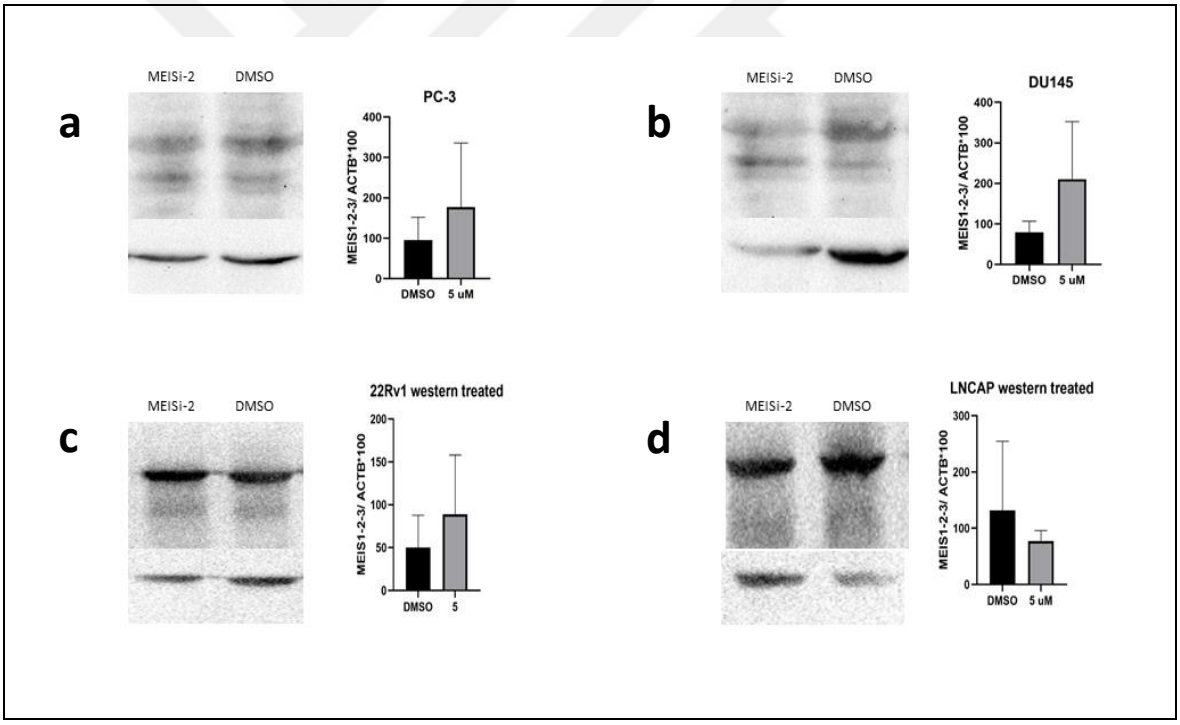


Figure 3.7. Determination of MEIS1/2/3 expression post treatment MEIS inhibitor-2. Representative images and bar graph of (a) PC-3, (b) DU-145, (c) 22Rv-1 and (d) LNCaP indicate the change of MEIS1/2/3 expression result from MEIS inhibitor treatment. The outcomes were analyzed by using Student t-test used and there was no any significant change.

3.6. MEISi-2 TREATMENT REDUCES EXPRESSION OF MRNA MEIS 1-2-3

PBX1-4 are co-partners of MEIS proteins in various biological process. Therefore, Pbx1-4 mRNAs expression levels were investigated post MEIS inhibitor treatment for 48 hours. Additionally, it is known that hypoxy inducible factors are targeted by MEIS transciption factor. Finally, Meis1/2/3 and related genes's mRNAs were investigated post MEIS inhibitor treatment.

For PC-3, all interest genes were downregulated resulted from MEIS inhibitor treatment. Compared to DMSO, Meis1 and Meis3 reduced 10-fold times and Meis2 were not detect via RT-PCR (Figure 3.8, a). After MEIS inhibitor treatment, Pbx1 and Pbx3 were downregulated four-fold and almost two-fold, respectively. However, Pbx2 and Pbx4's mRNA's level were not detectable (Figure 3.8, a). Hif1α and Hifα were not detecable and Hif2α expression were quite low, 80 percent the reduction of mRNA level (Figure 3.8, a).

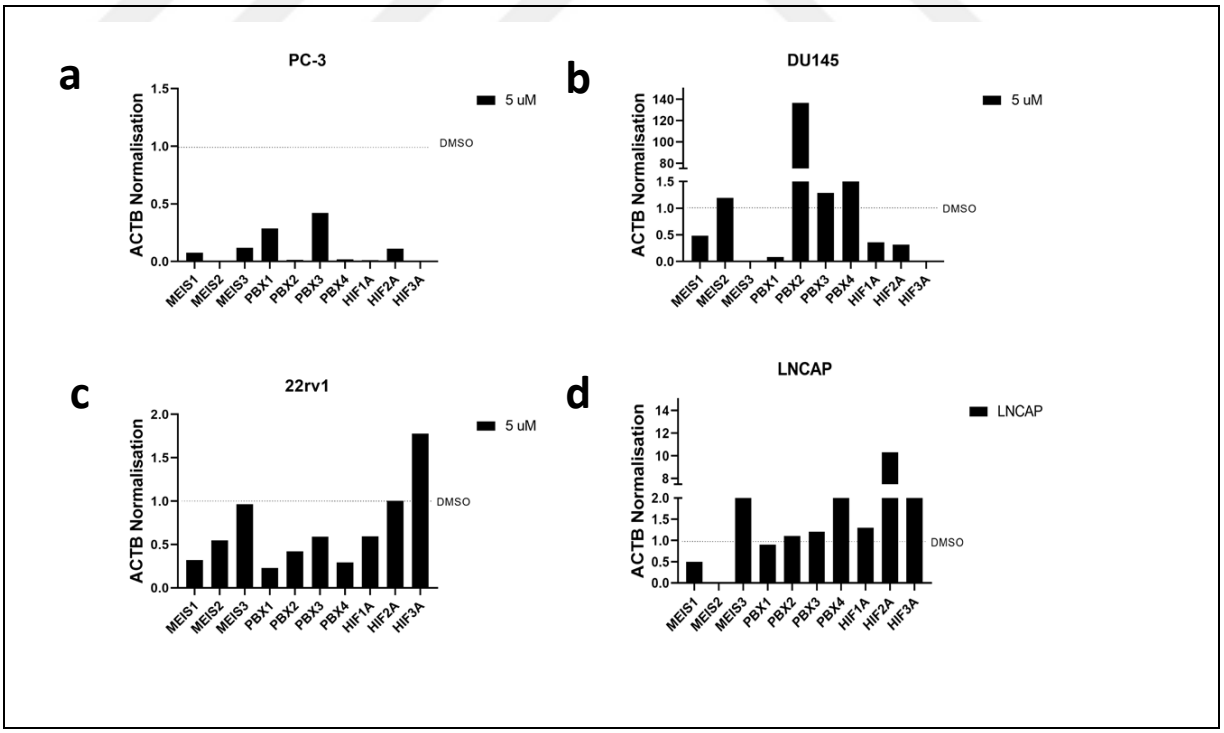


Figure 3.8. RT-PCR showing change amount of MEIS1, MEIS2, MEIS3, their co-partners (Pbx1, Pbx2, Pbx3 and Pbx4) and their target genes (Hif-1α, Hif- 2α and Hif3α) mRNA levels compared to each control groups in five uM MEIS inhibitor treated (a) PC-3, (b)

DU145, (c) 22Rv1 and (d) LNCaP for 48 hours. DMSO used for vehicle and the results analyzed with ΔΔCT method and eventually the results were normalized with ACTB. Discontinuous lines indicate mRNA of the interest genes for DMSO control.

In the DU145, Meis-2 expression increased post MEIS inhibitor treatment for 48 hours. However, Meis1 were downregulated to 50 percent. Also, Meis3 were not detecable. Pbx2-4 expression dramatically upregulated compared to DMSO. However, Pbx1 expression decreased from 1.0 to 0,1 (ten fold than DMSO). Despite the lacking of Hif3α, Hif1 and 2α reduced four-five fold compared to DMSO (Figure 3.8, b)

The reduction of Meis 1/2/3 had different proportion for 22Rv-1. Meis3 expression were as same as DMSO group. Meis2 and Meis1 reduced 0,5 and 0,3 of DMSO group's expression, respectively (Figure 3.8,c). Also, Pbx1-4 downregulated post five μM MEIS inhibitor treatment. After MEIS inhibitor treatment, Pbx3 expression reduce 50 percent. In addition, Pbx1, Pbx2 and Pbx4 reduced compared to DMSO (Figure 3.8, c). Although, there was no alteration for Hif2α, Hif3α upregulated post MEIS inhibitor treatment and Hif1α downregulated to 0,5 (Figure 3.8,c).

For LNCaP, Meis1 expression reduced to the half of DMSO and Meis3 expression were two-fold high than DMSO. However, Meis2 expression were not determined by using RT-PCR (Figure 3.8, d). Pbx1-3 expression were almost same to DMSO treated group, despite Pbx4 expression reached to 2- fold expression. The highest mRNA expression seemed to have Hif2α (eight fold expression). Hif3α and Hif1α expressed two-fold and 1,5 fold compared to DMSO (Figure 3.8, d).

3.7. MEISi-2 TREATMENT CAN LEAD IN INCREASE OF METASTATIC CAPABILITY

According to clinical studies, it is known that MEIS1-2 protein expression has an important place in the characteristics of prostate cancer. Therefore, it was checked whether the MEISi-2 treatment enhanced the metastatic properties of the PCa cells. As a result, the amount of cell migration increased compared to DMSO control as a result of suppression of the MEIS1-2-3 protein in parallel with clinical findings. This study, also known as the scratch assay, is widely used *in vitro* metastasis modeling (Figure 3.9.).

For PC-3, 1,25 μM MEIS inhibitor treatment reduced ten percent compared to DMSO and then 2,5 μM MEIS inhibitor slightly increased in regard of the relative cell migration of 1,25 μM MEIS inhibitor treatment. Ultimately, 5 μM MEIS inhibitor caused the increase of relative cell migration of PC-3 cell (Figure 3.9, a).

In DU-145, all MEIS inhibitor treatment led to the increase of relative cell migration compared to DMSO. The highest proportion of increasing relative cell migration were 20 percent with 1,25 MEIS inhibitor and then 2,5 and five μM followed to 20 percent increase, respectively (15 percent and 12-13 percent)(Figure 3.9, b).

There is no any change of relative cell migration proportion with the 1,25 and 2,5 μM MEIS MEIS treatment in 22Rv-1. However, five μM MEISi-2 increased relative cell migration from ten percent to 20 percent (Figure 3.9, c).

Although, 1,25 μM MEIS treatment increased relative cell migration of LNCaP, this effect reversed by 2,5 and five μM MEIS treatment. The result of five μM MEIS treatment, the reduction reached to 30 percent (Figure 3.9,d).

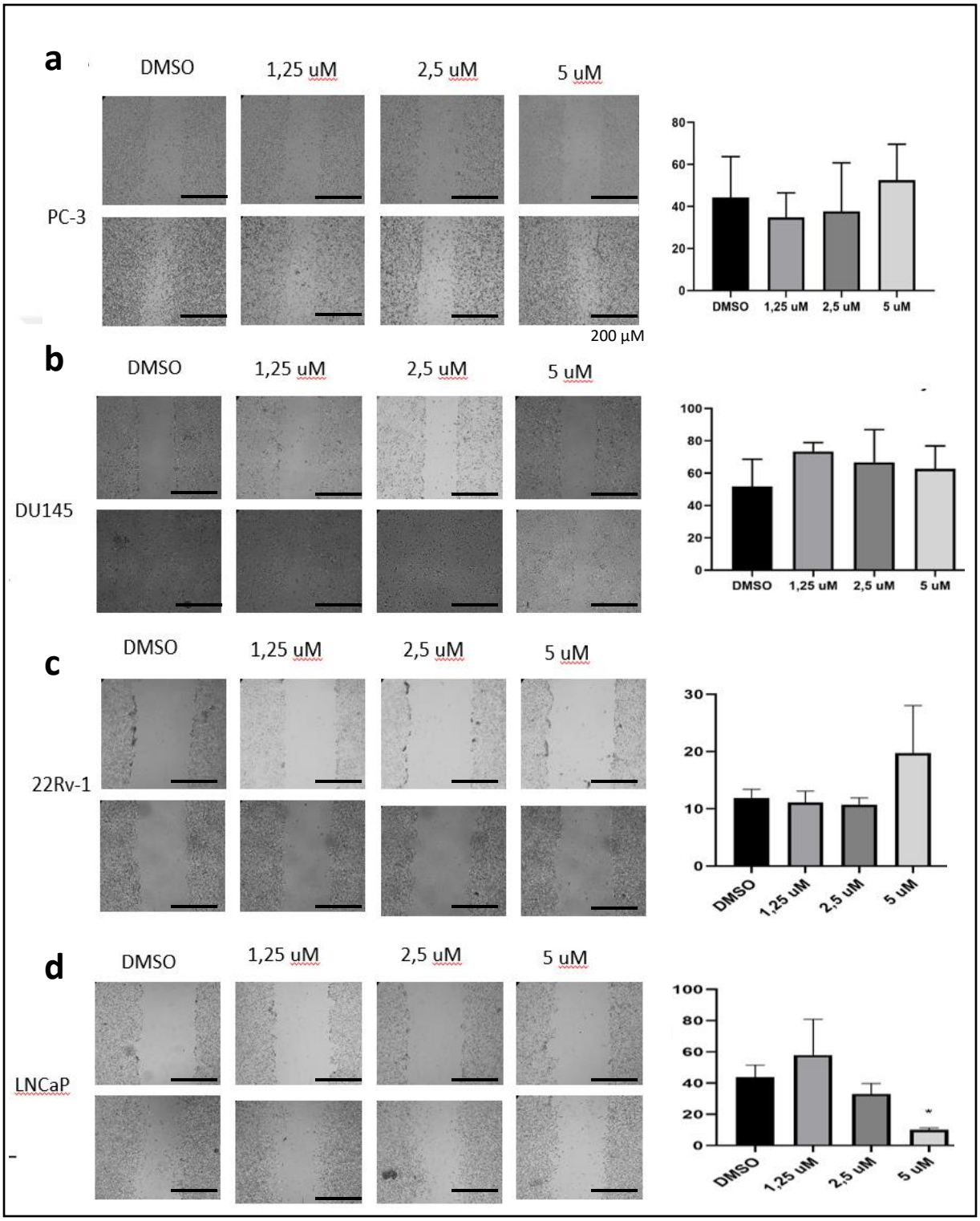
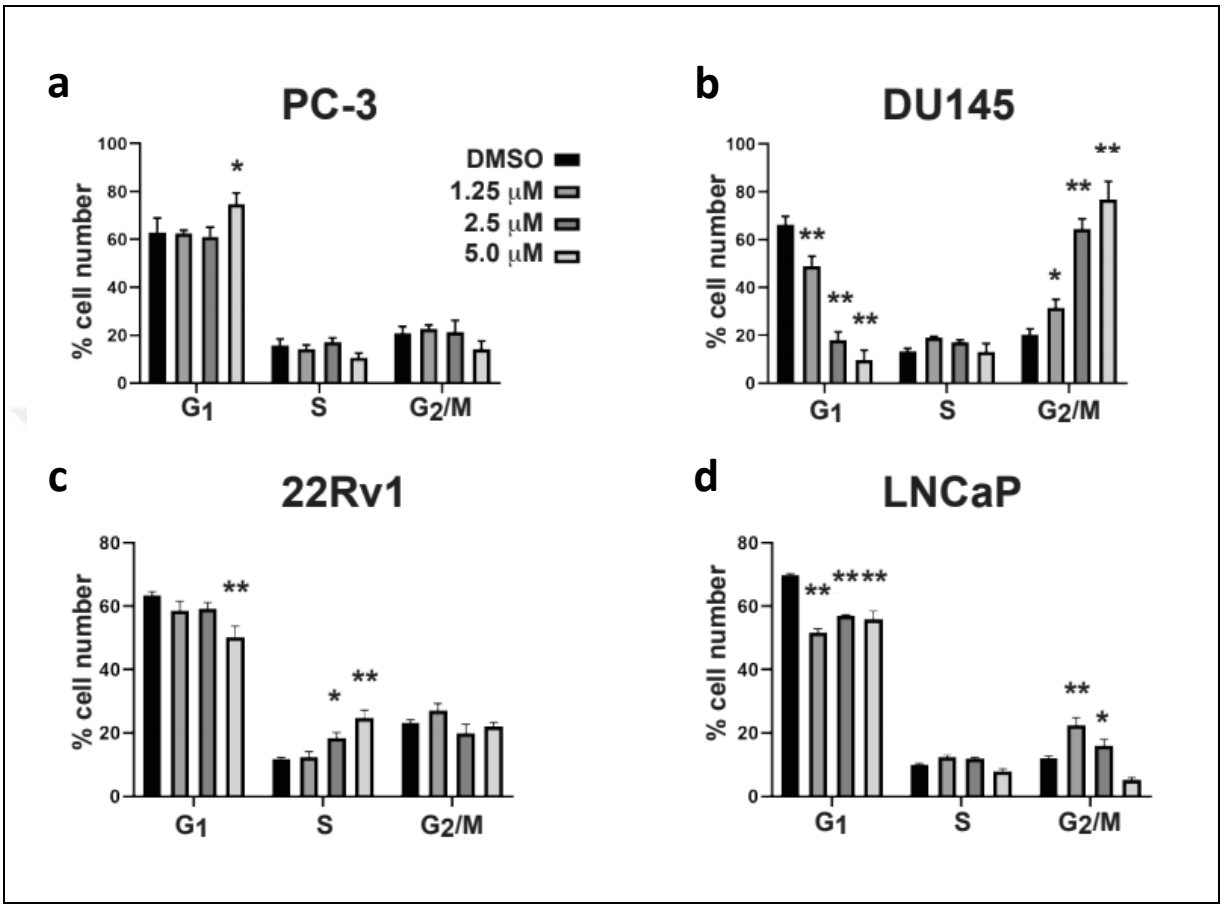


Figure 3.9. MEISi-2 treatment enhances PCa cell migration. Representative images and change of cell migration resulting from MEISi-2 treatment of (a) PC-3, (b) DU145, (c) 22Rv-1 and (d) LNCaP are shown in graphs, which setting the initial scratch as 100 percent. Bar graps

indicate that relative cell migration of Pca cell lines compared to DMSO control. All scale bars represent 200 μM. Student t-test used in order to determine P- values represented in the figure (n=3, *p<0.05).

3.8. MEIS1-2-3 PROTEINS CAN REGULATE THE CELL CYCLE

Effect on cell cycle regulation in Pca cell lines was investigated post MEIS inhibitor treatment. (Figure 3.10.). As a result of the five μM MEISi-2 treatment, accumulation of PC-3 in G1 increased by ten percent (Figure 3.10.,a). In DU145, G2/M arrest was increased in a dose-dependent manner as a result of 1.25 μM, 2.5 μM and five μM MEISi-2 treatment, with the shift of the cell population in G1 to G2/M by 20 and 25 percent, respectively (Figure 3.10., b). It is clear that 22Rv-1 and LNCaP have similar trend as a result of MEISi-2 treatment. MEISi-2 treatment clustered cell populations in G1 to S and G2/M phases for 22Rv-1 and LNCaP (5 and 25 percent, respectively(Figure 3.10.,c and d). Especially in LNCaP cells, some subpopulations other than G1, S and G2/M were formed during the MEISi-2 treatment. This may be due to DNA breaks due to MEISi-2 toxicity (Figure 3.10.,d, black arrows indicate subpopulations).


a
PC-3
DMSO
1.25 μM
2.5 μM
5.0 μM
% cell number
G1
S
G2/M
b
DU145
c
22Rv1
d
LNCaP

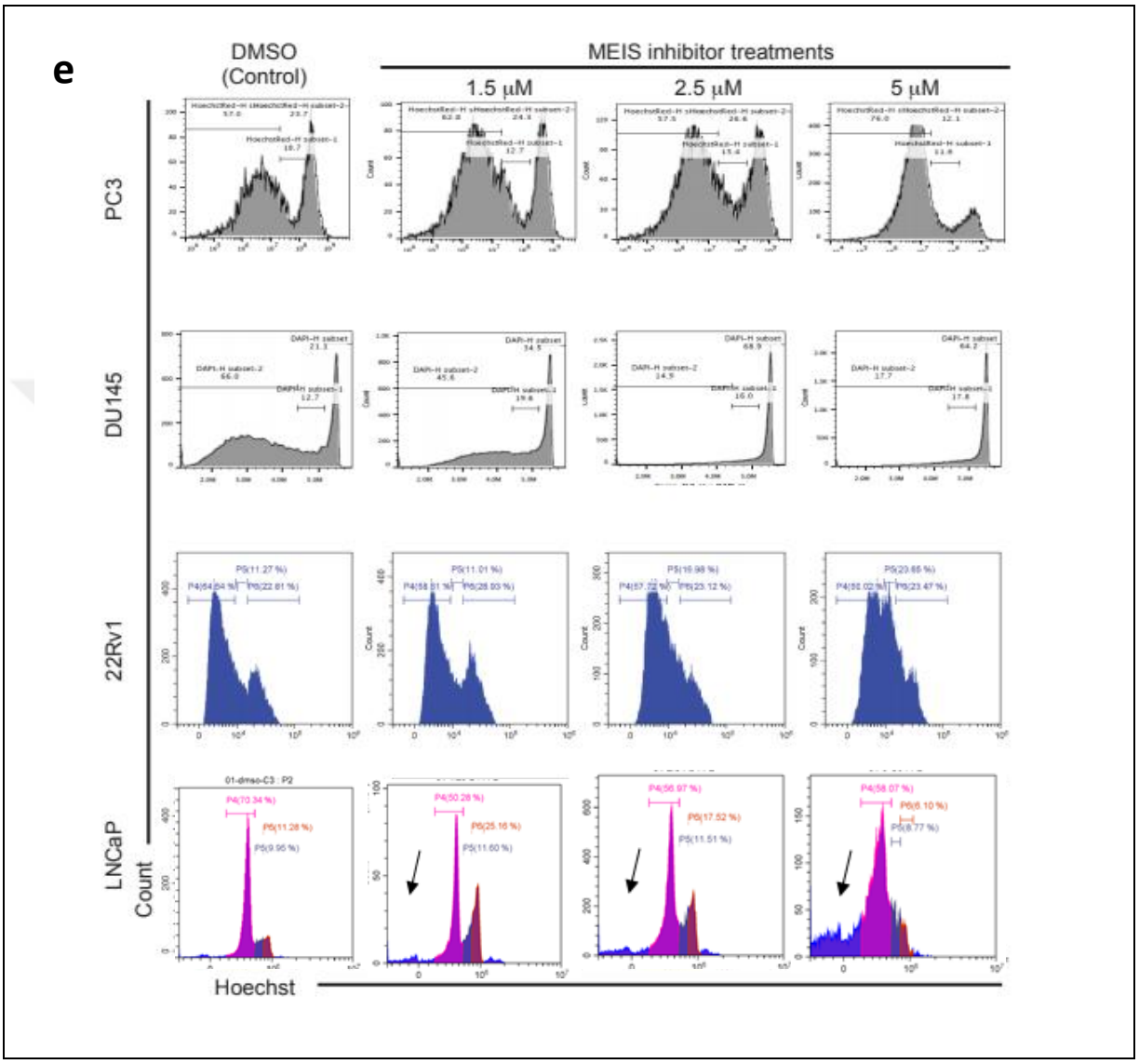


Figure 3.10. The effect of MEISi-2 on cell cycle in PCa cells. The shiffting/ cumulating of cell population and representative images of (a) PC-3, (b) DU-145,(c) 22Rv-1 and (d) LNCaP cells according to phases are shown. Representative images indicate the alteration of Pca cell lines post MEIS inhibitor -2 treatment (e). Student t-test used in order to determine P- values represented in the figure (n=3, *p<0.05)

3.9. MEIS1-2-3 CAN BE ESSENTIAL FOR PCA CELL SURVIVAL

Short-term MEISi-2 treatment of PCa cells enhances expression of mRNA and protein MEIS1-2-3. The hypothesis at this point is that PCa cells tend to increase MEIS1-2-3 expression in order to survive. Therefore, 15-day MEISi-2 administration was performed and the change in MEIS1-2-3 protein expression was investigated (Figure 3.11.). Deattached dead cells were removed from the culture medium and cells that could resist the short-term MEISi-2 treatment were sorted out. Consequently, the 15-day MEISi-2 treatment dramatically increased the amount of intracellular MEIS1-2-3 in PCa cell lines (Figure 3.11.). This dramatic increase can be attributed to the protein level in untreated cells. Considering MEIS1-2-3 expression in untreated cells, the intracellular MEISi-2 dependent increase is positively correlated (Figure 3.1 and Figure 3.11.).

After 2,5 μM MEIS inhibitor treatment, PC-3 and 22Rv-1's intracellular MEIS expression had 2- fold expression than DMSO control for 15 days (Figure 3.11, a and c). The gap between MEIS inhibitor and DMSO treated groups were higher than PC-3's gap. Intracellular MEIS expression of DMSO control were four fold lower than MEIS inhibitor treated DU-145 and LNCaP cells (Figure 3.11, B and d).

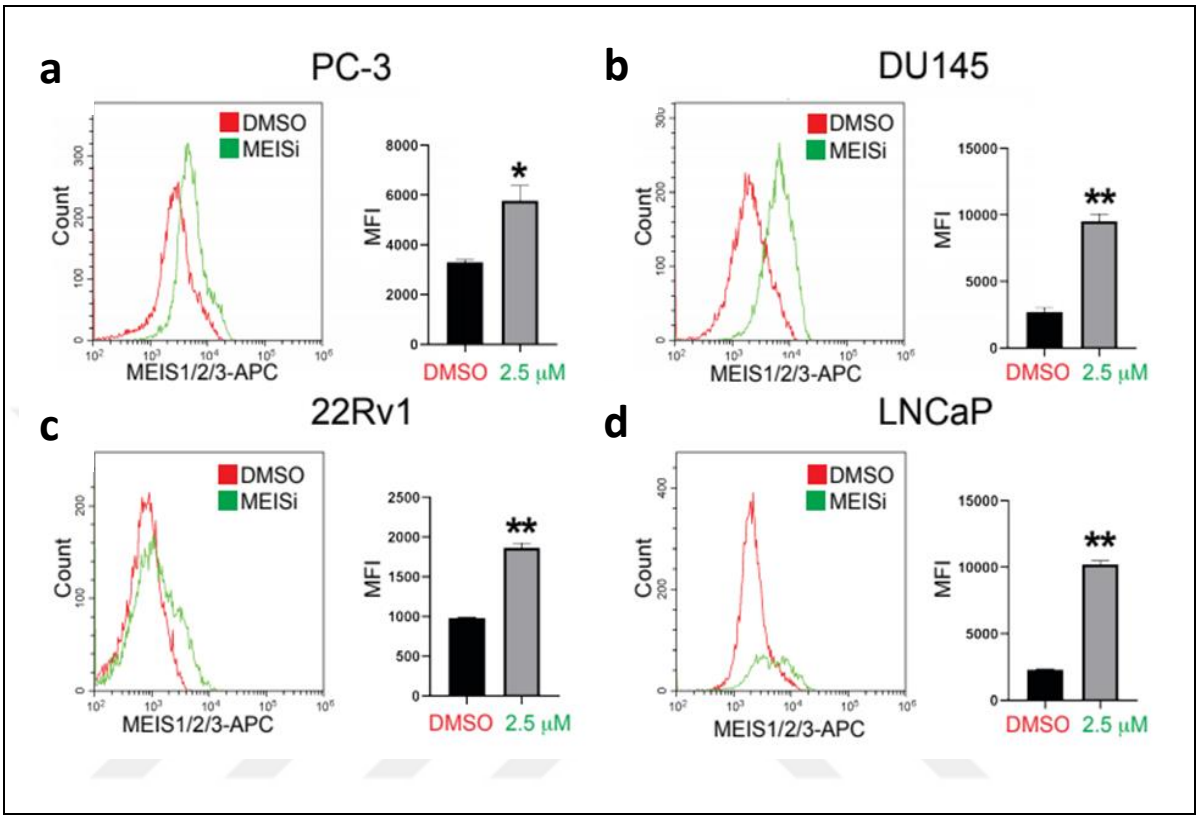


Figure 3.11. Flow cytometry analysis determines change of MEIS1/2/3 content in relatively long-term (15 days) MEISi-2 treated (a) PC-3, (b) DU145, (c) 22Rv1 and (d) LNCaP, respectively. Black bar represents MEIS1/2/3 expressin as a control; grey one indicate 2,5 uM MEISi-2 treated cells. Statiscally analysis was performed via using student t- test. (n:3, $p<0.05$, $**p<0.01$)

4. DISCUSSION

Since its discovery in 1995, MEIS proteins have been reported to have diverse roles in development, metabolism, homeostasis, regeneration and cancer biology[324]. The MEIS isoforms (MEIS1, MEIS2 and MEIS3) accomplish developmental and physiological processes in collaboration with HOX and PBX proteins[325]. It has been reported that cells undergo apoptosis as a result of impaired MEIS expression[13]. Expressions of MEIS and associated proteins are tightly regulated during development and adult tissues that contribute to overall homeostasis in the organism.

In the cancer biology, MEIS proteins were first described as an oncogene in the leukemia mouse model[324]. Over the last few decades, many studies have been conducted to understand the role of MEIS in leukemia compared to other cancer types. In recent years, the use of clinical data has demonstrated that MEIS and variying MEIS partners play crucial roles in development of various cancers, especially in neuroblastoma, cancers of male and female reproductive system [73, 261] (summarized in **Table 1.2**). Although MEIS was first described as an oncogene, a number of studies have demonstrated the tumor suppressor role of MEIS proteins as well [52, 80]. MEIS proteins could, for instance, suppress proliferation, migration, invasion and metastasis of renal cell carcinoma and gastric cancers [52, 80].

Our knowledge about MEIS and MEIS partner proteins are increasing gradually. The relationship of MEIS and MEIS partner proteins with various cancer types was discussed here as well as treatment strategies of these cancers with small molecules or non-coding RNAs (**Figure 4.1**). While the expressions of MEIS or its partner proteins in several cancer types are increased, they are decreased in many. Conventional chemotherapy often painful for cancer patients and, unfortunately, the success rate is limited. Therefore, researchers aim to develop targeted chemotherapy approaches. In a number of *in vitro* studies where MEIS and MEIS partner proteins are targeted indirectly by small molecules or non-coding RNAs, thus cancer proliferation, spread, metastasis, and invasion could have been suppressed. In conclusion, MEIS

and its partner proteins are key cancer biomarkers and could be therapeutically targeted in cancer treatments.

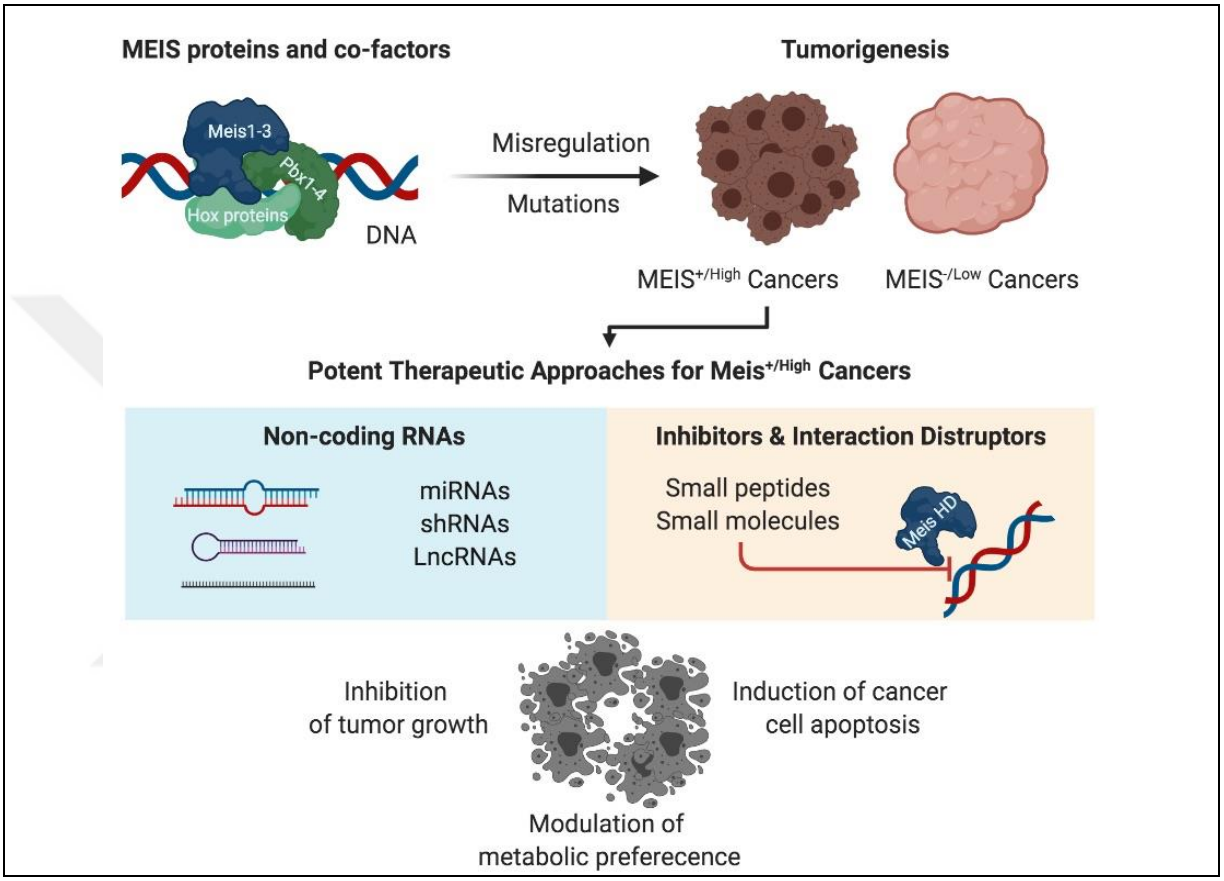


Figure 4.1. Potent therapeutic approaches for MEIS+/High cancers. MEIS1-3 and its cofactors are prominent oncogenic transcription factors. Various cancers could occur due to misregulation or mutations the MEIS transcription factors, which can be classified as MEIS+/High and MEIS-/Low. There are two types of potent targeted approaches for MEIS+/High cancers; RNA-based methods and inhibitors/disrupters. While RNAs-based methods inhibit the translation of MEIS1-3 transcripts, the disruptors bind of MEIS protein in order to block its function or interaction with other cofactors. Thus, these approaches may cause inhibition of tumor growth, induction of cancer cell apoptosis, and modulation of metabolic preference.

PCa is the second most common cancer in the males. PCa with local benign masses are less risky than aggressive and metastatic ones. In this study, the efficacy of MEIS homeodomain inhibitors on PCa cell lines, previously developed in our laboratory as a first-in-class, was examined. A seriously negative correlation was detected between MEIS expression and MEISi-2 IC50 values of PCa cell lines. Within 48 hours, MEISi-2, the MEIS homeodomain inhibitor, suppressed the cell proliferation of PC-3, DU145, 22Rv-1 and LNCaP cells and increased significantly the amount of apoptosis in the cell population. On the other hand, within 48 hours, Meis1-2-3 and its cofactors Pbx1-4 and Hif 1-3 mRNA expression to target genes were down-regulated in all treated PCa cells. In addition, MEISi-2 cells also increased sharply the level of cellular ROS.

Interestingly, there was no positive correlation between the amounts of untreated Meis1-2-3 mRNA and MEIS1-2-3 protein. Some previous studies have proved that there may not be a numerical relationship between mRNA and protein levels, especially in cancer cells[326]. It has been revealed that post-transcriptional modification may be more important than mRNA level in determining the protein level[327]. The importance of alternative splicing methods in terms of protein abundance has been demonstrated than the amount of mRNA[328]. In contrast, there are studies claiming that mRNA level is a measure in order to predict protein level [329, 330]. The process from mRNA to protein has not yet been fully elucidated, and this study has not provided data on the estimation of the protein level of the MEIS1-2-3 via analyzing mRNA level in PCa untreated cells.

In previous studies, there is a small-molecule based on inhibitor with a sensitive effect due to the low MEIS1 expression[318]. Bhanvadia showed that the importance of MEIS 1-2 expression in PCa, it has been shown that high MEIS1-2 expression is essential for increase local tumor mass [331]. As a MEISi-2 targeted inhibitor for MEIS active site, it is more sensitive to high MEIS1-2-3 protein and has a lower IC50 value, and vice versa (Figure 2). As a result, MEISi-2 may be a potential chemotherapeutic agent in PCa's where there is an oncogenic MEIS expression.

Many studies in leukemia have demonstrated oncogenic MEIS1-2 protein expression causes apoptosis or proliferation suppression in leukemic cells, it was observed when MEIS expression was down-regulated [332]. On the contrary, the number of studies providing evidence of oncogenic MEIS1-2 expression in PCa is few[221, 333]. It was shown for the first time in this study that inhibition of MEIS1-2-3 in PCa cell lines leads to apoptosis. Regarding of the increase ROS, most studies demostrated apoptosis in PCa [334, 335]. ROS increase due to MEISi-2 treatment is considered one of the strongest causes of apoptosis in this study.

It also appears that the PCa cell lines show a resistance to MEISi-2 treatment. MEIS1-2-3 proteins increase in cells after MEISi-2, which is treated to prevent the binding of MEIS1-2-3 homeodomain to DNA (Figure 3.5 and 3.5.1). On the contrary, MEISi-2 treatment sharply suppresses the expression of Meinox mRNAs. Leila Dardai et all reported that this is caused about on and off switching system the promoter regions due to the amount of MEIS1-2-3 transcription factor [336]. Probably the inhibition of MEIS1-2-3 to bind to DNA caused MEIS-mediated pathway to be silenced in PCa cells, while the slightly protein increase is related to the Meis1-2-3 mRNA already present as a result of post-translational modifications. MEIS mediated pathway consist some regulation related to metabolic activity, metastasis/angiogenesis and aggresiveteness of Pca [337].

As is clearly known, local prostate cancer and metastaic-aggressive PCa exhibit significantly different clinical features[338, 339]. Therefore, we examined the metastatic capability of PCa cells as a result of MEISi-2 treatment by scratch assay. Increased cell migration resulting from MEISi-2 treatment confirms previous studies for PCa[331]. Cell migration slightly increases as a result of the low-dose MEISi-2 treatment.

Previous studies on how MEIS1 regulates the cell cycle in solid tumors are not sufficient. Over-expression of MEIS1 in gastric cancers and renal cell carcinomas cells causes arrest at the G1 / S transition point[80, 340]. In contrast, MEIS1 inhibits cell cycle entry in non-small lung cancer cells and has a tumor suppresor feature. Although there are very few studies investigating the effects of MEIS1-2-3 on the cell cycle in solid tumors; MEISi-2 treatment lowers the proportion of cells in G1 phase, indirectly confirming the upregulation of MEIS1 results in gastric and renal

cell carcinoma. In this study, it show that MEIS inhibitors can be a candite chemotherapeuric agent for Pca. MEISi-2, a small molecule based MEIS inhibitor, is able to reduce cell viaility of Pca cell lines, PC-3, DU-145, 22Rv-1 and LNCaP. Also, MEISi-2 treatment led to the increase of ROS and MEIS protein expression although MEIS1-3, PBX1-4 and Hif1-3α mRNAs levels were downregulated post MEIS inhibitor treatment.

In conclusion, we have developed a original small molecule, MEISi-2, that rapidly show cytotoxic effects in a PCa cell lines, PC-3, DU145, 22Rv-1 and LNCaP. To the best of our knowledge, this is the first time druggable MEISi-2 target MEIS1-2-3 proteins in PCa cell lines. Identification of the underlying mode of action of the compound will allow the possible approaches of PCa chemotherapy with oncogenic MEIS1-2-3 selective, targetable MEINOX pathway that could have important clinical impact for patients who suffer from this common disease.

5. CONCLUSION

For last decades, literature showing that MEIS proteins are tumor suppressor and oncogenes have been revealed. Accordingly, the evidence that MEIS proteins can have an important role in cancer biology continues to increase rapidly.

In terms of PCa, studies with clinical samples have generally shown that MEIS proteins have different expression levels depending on whether they are benign (local) or metastatic tumors (aggressive). As a result, MEIS proteins can be vital for PCa and need to be expressed at every stage of prostate cancer.

In this study, MEISi-2, MEIS inhibitor, which was developed for the first-class, was proven to inhibit MEIS by *in vitro* experiments by metabolic pathways. MEISi-2 decreased cell viability of PCa cells and has been observed to trigger their apoptosis. Also, as a result of MEISi-2 treatment, MEIS1-2-3 protein expression was increased in PCa cells and these MEIS1-2-3 up-regulated cells were able to survive. This study is the first finding that MEIS1-2-3 protein expression may be essential for PCa cells.

6. FUTURE WORKS

In the future,the effect of small molecule based MEIS inhibitors should be validated by some RNA technologies. It is known that siRNA, miRNA and LncRNA are conventionally used for the interest protein inhibition. Although, the RNAs are not as appropriate as small molecules for drug development because of the lacking stability. At least, the outcomes of MEISi-2 effects could be validated by RNAs technologies.

Also, MEIS inhibitors were dissolved in DMSO. It is known that DMSO is not a convenient vehicle agent for using the clinical approaches due to its toxicity.In our study we showed that the solubility of MEISi-2 in the solvent with high pH values (data not shown) because of probably becoming salt. In this point, the effectiveness of MEISi-2 in solvent (high pH) should be investigated and then more convenient formulation should be developed via using pharmaceutical techniques.

Additionally, some non—orthotopic xenograft models were modelled in the master thesis (PC-3 and 22Rv-1 for Pca). These studies should be improved with orthotopic models and patient derived models in regard to MEIS expression. Because non-orthotopic models could be insufficient in order to mimic cancer diseases.Although MEIS expression seems to be negatively correlated with the stage levels of Pca, with the neuroendocrine phase MEIS expression was increased by the time. Therefore, MEIS inhibitors could be effective against CRPC cells with other chemotherapies approaches. Firstly, the clinical samples of patients suffering from Pca, should be collected and then cells from these tissues should be isolated in order to determine MEIS expression. Ultimately, the effectiveness of MEIS inhibitors could be investigated and validated by *ex vivo* cell culture.

Finally, tracking is a really important issue for drug development studies.There is no adequate knowledge related to delivery and circulation of MEIS inhibitors body. Therefore, the determination and tagging of MEIS inhibitors after the treatment should be devised in order to understand the effects of MEIS inhibitors on whole biological systems.

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