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YY1 and NFYA: Potential tr-KIT Specific Transcription Factors in Prostate Cancer

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Abstract

Objective: Via the use of an alternative promoter, a truncated c-KIT protein (tr-KIT) of 30-50 kDa is generated, lacking extracellular and transmembrane domains. Moreover, over-expression of tr-KIT, a stronger activator than c-KIT, appears to be specific to prostate cancer (PCa). Also, Imatinib, a tyrosine kinase inhibitor, blocks the activity of full-length c-KIT but has no effect on tr-KIT in PCa. Tr-KIT has its own nuclear factor binding site. However, the transcription factors (TFs) binding to this region specific to tr-KIT are not known yet. This study was conducted to define the most potential TFs specific for tr-KIT via in silico analysis.

Methods: Tr-KIT potential TF binding sequence was uploaded into Tfsitescan database. Five TFs with the highest potential binding to this sequence were selected. Transcriptomic data of LNCaP (PCa expressing tr-KIT), PC3 (PCa not expressing tr-KIT) and RWPE-1 (normal prostate) cell lines (GSM1647378, GSE36022 and GSM738189, respectively) from Gene Expression Omnibus (GEO) database were compared for gene expression levels of pre-defined potential tr-KIT specific TFs using DESeq package of R-program. Finally, two TFs having higher expression levels in both LNCaP and PC3 compared to RWPE-1 and higher expression levels in LNCaP compared to PC3 were detected.

Results: Five TFs having the highest potential were selected as: YY1, c-MYB, IL8, NFYA and TCF3. Via in silico analysis performed, it was found that YY1 and NFYA have the highest potential to be tr-KIT specific TFs in PCa, among them.

Conclusion: YY1 and NFYA TFs may take a role in formation of tr-KIT in PCa.

Key words: Prostate cancer, transcription factors, gene expression regulation

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INTRODUCTION

Prostate cancer (PCa) is the most widespread internal organ tumoral formation in men and the second most widespread type of cancer worldwide (1). C-KIT (CD117) is an oncogenic receptor tyrosine kinase overexpressed in PCa cases. Moreover, c-KIT regulates cell proliferation in the prostate and plays an important role in the pathophysiology of benign prostatic hyperplasia (BPH). In addition, c-KIT expression in PCa patients has been found to correlate with disease relapse. That provided c-KIT to be considered as a choice for anti-cancer therapies. Currently, inhibitors of c-KIT interfering with c-KIT signaling pathways are options for the treatment of PCa, gastrointestinal cancers and leukemia, and in the assessments of other cancer types (2). Via an alternate promoter, a truncated c-KIT protein (tr-KIT) of 30-50 kDa is formed, not having the transmembrane and extracellular domains (3). Tr-KIT is a stronger activator of Src kinases in comparison with the full length c-KIT protein. Higher Src efficiency was detected in PCa tissues and cells expressing tr-KIT. The markedly increased transcriptional activity of tr-KIT with PCa aggression provides it to be considered as a pharmacological target (4, 5).

Imatinib is a tyrosine kinase inhibitor that has the ability to slow and stop tumor formation by targeting the extracellular part of c-KIT, which is overexpressed in PCa cases, by suppressing it. Imatinib arrests the efficiency of full-length c-KIT however doesn't have influence on tr-KIT. This explains the ineffectiveness of this agent in the therapy of PCa, and the discrepancy between in vitro test results and clinical findings. Moreover, vascular endothelial growth factor (VEGF), an angiogenesis factor, represented a marked reaction to Imatinib in DU145 and PC3 cells. Imatinib treatment reduced VEGF expression levels in DU145 cells, while an opposite effect was observed in PC3. Those adverse influences of Imatinib in different cell lines might conduce to explain the lack of efficiency of that anti-tumor agent to control prostate tumor development and increase anxiety about the induction and progression of metastasis in case of administering imatinib therapy in PCa patients. As a result, the transcriptional activities of tr-KIT and c-KIT in PC3 and DU145 cell lines identify the variable influences seen in response to Imatinib with decreased full-length c-KIT expression and rised tr-KIT expression. Overall, the available information and the data obtained above support more comprehensive studies aimed specifically at defining agents blocking tr-KIT (6). So, it is crucial to understand tr-KIT activation mechanism in PCa.

Tr-KIT possesses its own transcription factor binding region (a site in the 16th intron of the c-KIT) (4, 7). Even so, transcription factors (TFs) targeting to that site particular to tr-KIT are not defined still. This study was performed to define the most probable TFs in silico by analyzing the potential TF binding site for tr-KIT

METHODS

Sequence based potential TF analysis

Firstly, we have to mention that this is an in silico study so there is no need for Ethics Committee Approval.

Potential TF binding sequence specific to tr-KIT was loaded into database of Tfsitescan (8). The nucleotide sequence of tr-KIT-specific possible TF binding region in the 16th intron of c-KIT gene (NC_000004.12) is as follows:

>gi|568815594|ref|NC_000004.12|:54732094-54732264 Homo sapiens chromosome 4, GRCh38.p12 Primary Assembly

5'-
TAGTTAAAATGCAGAATGTCATTTTGAAGTG
TGGTAACCAAAAGCAGAG
GAAATTTAGTTTCTTCATGTTCCAACTGCTGT
CTCTTTGGAATTCCTGTTCTAATTTATAAGCT
GTAAAGTACAAGCCTGTCTAAATGAGTTTTT
CTATGAATATTCTTTTATATGCAGTGA-3'

Five TFs with the highest binding probability to that region were chosen.

Comparison of transcriptomic data and statistical analysis

From Gene Expression Omnibus (GEO) database, transcriptomic data of LNCaP (PCa expressing tr-KIT), PC3 (PCa not expressing tr-KIT) and RWPE-1 (normal prostate epithelium) cell lines (GSM1647378, GSE36022 and GSM738189, respectively) were compared with respect to gene expression levels of five pre-defined potential TFs using DESeq package of R-program. For differential gene expression analysis, the number of reads mapped to each pre-defined potential TFs was calculated and presented utilizing the HTseq package, and the output data was processed as input data for statistical computations. The Q-value obtained from statistical approaches were utilized to assess gene expression differing between cell lines (9).

Consequently, this analysis presented that two TFs with higher transcriptional activity in both PC3 and LNCaP in comparison with RWPE-1 and with higher transcriptional activity in LNCaP in comparison with PC3 were obtained

RESULTS

Having the highest potential binding to tr-KIT-specific possible TF binding region sequence according to sequence based potential TF analysis, five TFs selected as follows: c-MYB, YY1, NFYA, TCF3 and IL8.

As a result of in silico comparsion of LNCaP (PCa expressing tr-KIT), PC3 (PCa not expressing tr-KIT) and RWPE-1 (normal prostate epithelium) cell lines' transcriptomic data (Figure 1), we detected only Yin Yang 1 (YY1) and Nuclear Transcription Factor Y Subunit Alpha (NFYA) TFs provided the condition with higher transcriptional activity in both LNCaP and PC3 in comparison with RWPE-1 and with higher transcriptional activity in LNCaP in comparison with PC3. So, YY1 and NFYA TFs defined to possess the highest potency to become TFs particular to tr-KIT.

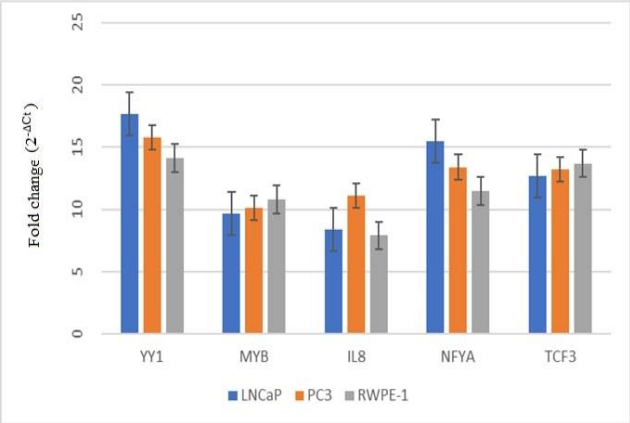

Figure 1. Comparison of transcriptomic data

DISCUSSION

Tr-KIT is a strınger activator of Src kinases in comparison with the full length c-KIT protein. Higher Src efficiency was detected in PCa tissues and cells expressing tr-KIT. Tr-KIT activation mechanism is very important to understand PCa and imatinib resistance better. Yet, tr-KIT specific TFs are not known. In this study, we defined two TFs with the

highest probability to become tr-KIT specific TFs, YY1 and NFYA. This shows potential oncogenic roles of YY1 and NFYA for PCa as activators of tr-KIT. As presented below, the findings related with YY1 and NFYA with respect to their potential to activate PCa are so convincing.

Heterogeneous nuclear ribonucleoprotein M (hnRNPM) can bind to cis-acting elements or various proteins and implement variety of biological functions in different cells. HnRNPM has a lower transcriptional activity both in PCa tissue compared to benign prostate hyperplasia (BPH) tissue and in neuroendocrine prostate cancer (NEPC) tissue compared to adenocarcinoma tissue. Yang et al. showed that YY1 overexpression might trigger epithelial-mesenchymal transition (EMT) by decreasing hnRNPM transcriptional activity in PCa cells (10). Also, XAF1 having absent or low transcriptional activity in cancer is a tumor suppressor gene product. Ectopic-mediated expression or transcriptional reactivation of XAF1 suppresses cancer progression. Camacho et al. reported that YY1 could suppress tumor suppressor gene XAF1 through HDAC1 downregulation or inhibition and because of mutation at the YY1-binding region in XAF1 promoter in PCa (11). Moreover, the high expression of miR-146a affects PCa cells with respect to progression, viability, and apoptosis. Huang et al. expressed that YY1 depletion suppressed PCa cell proliferation and viability, and triggered apoptosis via miR-146a assistance (12).

Protein arginine methyltransferase 5 (PRMT5) add methyl groups to non-histone protein substrates and arginine residues of histones in parallel and mediates various cellular functions via epigenetic control of post-translational modification of signaling molecules or target gene expression. The latest evidence proposes that PRMT5 can serve as an oncogene and its excessive expression causes the formation and progression of many different human cancers. Zhang et al. found that PRMT5 regulated the effect of NFYA to activate cell grown in LNCaP prostate cancer cells (13). Also, Chan et al. indicated that the phosphorylation of NFYA regulated the transcriptional expression of G2 checkpoint mediators (cyclin B1, cyclin A2, cdc2, and cdc25c) through binding to CCAAT motif in their promoters in PCa cells (14).

These studies support our findings giving potential oncogenic roles to YY1 and NFYA and increasing their clinical significance for PCa as activators of tr-KIT.

Some limitations of this study should be noted. First, tr-KIT potential TF binding sequence was used to determine tr-KIT specific TFs (4, 7). However, there may be other genomic regions that have not been identified as tr-KIT specific TF binding site yet. So, we can just analyze the region identified up to now. Second, we defined potential tr-KIT specific TFs, YY1 and NFYA, upon transcriptomic data of PCa cell lines. But, for now, we don’t know their validity in PCa cancer patients although it is within our future plans.

CONCLUSION

All in all, YY1 and NFYA TFs might take a function in production and activity of tr-KIT in PCa and provide clinically significant information about PCa progression and Imatinib resistance via tr-KIT activation. More universal research is required to figure out the functions of NFYA and YY1 TFs in PCa progression via higher tr-KIT transcriptional activity.

Ethics Committee Approval: Clinical Studies Ethics Committee of Ordu University, Faculty of Medicine was not needed.

Peer-review: Externally peer-reviewed.

Author Contributions:

Concept: S.E., Design: S.E, F.A.; Literature search: S.E., D.U.A, Data Collection and Processing: S.E. F.A., E.B., D.U.A., T.N, H.E., Analysis or Interpretation: S.E., F.A., D.U.A, Y.K.A., Writing: S.E.

Conflict of Interest: No conflict of interest was declared by the authors.

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