Molecular mechanisms underlying the role of Tumor protein D52 (TPD52) in the Prostate cancer progression

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Summary of the work done

Title of the work:

Molecular mechanisms underlying the role of Tumor protein D52 (TPD52) in the Prostate cancer progression.

Introduction:

Prostate cancer (PCa) is the most leading cause of deaths worldwide after Lung and Colorectal cancers. One in nine men is diagnosed for PCa after the age of 60 in the western world. PCa arises due to inheritance, food habits, culture, environmental causes, and age. When cancer cells morph to become sophisticated enough to move beyond the prostate, they learn evasive maneuvers. Evolution of drug-resistant mechanisms and recurrence of tumors vitiate current therapeutic approaches. These conditions necessitate the increased understanding of oncogenes responsible for PCa and its progression. Oncogenes arise due to the accumulation of mutations due to genetic and conditional factors which are essential to study in order to provide advanced therapeutic solutions. Tumor Protein D52 (TPD52) is among the oncogenes which frequently overexpressed due to gene amplification in Breast cancer, PCa and Colon cancers. *In vitro* studies on TPD52 evidenced that it intervenes in Lipid droplet formation, Vesicle trafficking, Calcium-dependent signaling, Cell cycle regulation, Docetaxel drug resistance, Migration, Invasion, and Proliferation. These versatile mechanisms of TPD52 make it as an essential gene of study, in cancer progression. Especially in PCa, TPD52 was reported to be overexpressed by Robert Bright et al. in 2004 and Ummanni et al. in 2008. There are various reports suggesting the oncogenic functions of TPD52 in PCa, but the detailed mechanisms responsible for them remain elusive.

To untangle the TPD52 association with oncogenic pathways, we have carried out research through which we found its role in alteration of the proteins which are members of the NF-κB pathway. We also found the transactivation of NF-κB and STAT3 by TPD52 overexpression. In addition to this, we have performed a proteomic-based study of TPD52-interactome which evidenced us TPD52's novel function in regulating the oxidative stress during cancer progression. In order to perform experiments to define TPD52's functions *in vitro*, we used two PCa cell lines, i.e., androgen-dependent LNCaP cells and androgen insensitive PC-3 cells in as the major tools in our experiments.

Objectives of the study:

1. Studying the altered mechanisms of prostate cancer cells due to aberrant TPD52 expression during prostate cancer progression.
2. Identification of novel interaction partners for TPD52 (*Interactome*) through a proteomic approach.

Methodologies and Results:

1. Studying the altered mechanisms of prostate cancer cells due to aberrant TPD52 expression during prostate cancer progression.

To find the novel roles of TPD52, in cancer progression we have carried out *in silico* analysis using online bioinformatics tools like Cytoscape, Genemania, String, and others. *In silico* analysis of TPD52 and TPD52's interacting partners, structural and functional interactions showed more extensive network through its family members. One of the subnetworks contains NF-κB pathway proteins, p65, and Ik-Bα which are associated with cancer progression by defining the tumor microenvironment through generation pro-inflammatory markers. Based on this analysis, we measured the p65 NF-κB promoter reporter assay by luciferase activity. Interestingly, TPD52 overexpression was able to induce p65 promoter, but the downregulation fails to do so. While the altered expression of TPD52 exogenously, showed aberrations in proliferation and cell cycle regulation. TPD52 upregulation increased the rate of cell proliferation in LNCaP cells increased while the downregulation suppressed the cells proliferation. Overexpression of TPD52 augmented the LNCaP cell growth might be through increasing cyclinD1, Bcl2, XIAP protein levels; whereas the downregulation of it leads to decreased cell proliferation could be by the induction of pro-apoptotic factors Bax, $p27^{Kip1}$ and cleavage of PARP. We pursued the study to find whether these oncogenic functions are mediated by the NF- κB pathway using western blotting, ELISA and other molecular techniques. Whereas, the TNFα-induced p65NF-κB activation was also be nullified by downregulation of TPD52. It proved that TPD52 could become a crucial factor for NF-κB activation in PCa. Further study on TPD52 reveals that it can regulate the expression of matrix metalloproteinases, cytokine secretion, and cell adherent proteins thereby contributing to tumor progression. We found that the transactivation of STAT3 was mediate by NF-κB in LNCaP cells by

using the Stattic and parthenolide drugs which inhibits the activation STAT3, NF- κB respectively.

2. **Identification of novel interaction partners for TPD52 (*Interactome*) through a proteomic approach.**

TPD52 involved in versatile functions has been reported *in vitro*. It might regulate different pathways by forming homodimers and/or heterodimers owing to having its water soluble and flexible structural motifs. In order to study its novel functions, we destined to study its interacting partners by pull-down assays coupled with the proteomic approach. TPD52 binding proteins were pulled down by the recombinant TPD52 tagged with GST. Immobilized proteins were subjected to 2- Dimensional gel electrophoresis followed by identification of differentially bound proteins by using LC-MS/MS coupled with *in silico* analysis and peptide search in MASCOT peptide database. Identified proteins contain already reported interacting partners along with novel interacting partners. Upon bioinformatics analysis of Protein interaction networks using identified proteins, as input, we found a variety of protein-protein interaction networks depicting different signaling pathways. One of the proteins is Peroxiredoxin 1 which is a novel interacting protein, connecting TPD52 with Redox signaling mechanism which may assist us to decipher TPD52 role in counteracting the increased oxidative stress, during enhanced migration and proliferative processes.

TPD52 interaction with PRDX1 was validated using *in vitro* pull-down assays followed by functional characterization. In cell-free peroxidase assays using pyrogallol, we found that TPD52 presence could increase the peroxidase activity of PRDX1. The retroviral-mediated stable expression of TPD52 in PCa cells, provided evidence that the TPD52 enhances the dimerization of PRDX1, thereby effecting the detoxification of peroxides. The conditional expression of TPD52 using doxycycline-dependent Tet-On system reciprocated our assumption about dimerization of PRDX1. Apart from this, we found, the interaction was dependent on oxidative stress where we found increased binding of PRDX1 with NTAP-TPD52. In addition, the accumulation of peroxides increased due to the combined downregulation in the cells evidencing that TPD52 interaction with PRDX1 is essential to counteract oxidative damage in cancer cells. In our effort to map the region of TPD52 binding with PRDX1, we found that the C-terminal region of TPD52 plays crucial role interest. In addition, we found the positive correlation of their combined expression with

poor patient survival in clinical samples data obtained from The Cancer Genome Atlas (TCGA) which makes our study important by deciphering the novel interaction of TPD52 with PRDX1.

cell growth was supported by inhibition of cell cycle due to elevated $p27^{Kip1}$, a cell cycle inhibitor and decreased antiapoptotic signal Bcl-2 expression. This observation hypothesizes that PRDX1 and TPD52 complex play a role in the survival of PCa cells evading apoptosis.

Frequent aberrant expressions of TPD52 and PRDX1 is reported in multiple cancers [46, 48, 104, 128, 134, 152, 162]. To understand the expression pattern of TPD52 and PRDX1, analysis of gene expression data on PCa from TCGA database showed a positive correlation between TPD52 and PRDX1 expression. Further, the higher expression of TPD52/PRDX1 affected the overall survival of high-grade PCa patients. Consequently, elevated TPD52 and PRDX1 in PCa patients may interact with each other and this interaction is colligated with the aggressiveness of PCa thereby poor patient survival.

4.6 Conclusion:

In conclusion, this study has reported novel candidate partner PRDX1 for TPD52 overexpressed in prostate cancer by GST pulldown assay coupled with 2DE and *in vitro* validation. Through the functional characterization of the interaction, we identified that the TPD52 ability to promote peroxidase activity of PRDX1. Functionally, TPD52 induced increased peroxidase activity of PRDX1 might be indulging in mitigating the cellular damage due to higher oxidative reservoirs in cancer cells (survival) or in maintaining the higher oxidative capacity within cancer cells (aggressiveness). In our attempt to find the PRDX1 interacting domain with the TPD52, we found the C-terminal region was crucial in the binding of these two proteins. In addition to this, the migratory potential was increased in PCa cells with combined overexpression, which infers us that the interaction might play an important role in developing the aggressiveness of PCa cells. Our understanding of clinical data obtained from The Cancer Genome Atlas (TCGA) through web tools, supported the positive correlation of co-expression (Pearson correlation coefficient value=0.35) and the importance of the interaction during the development of aggressive phenotypes. Be of the significance of TPD52 and PRDX1 interaction in *in vitro,* and it is noteworthy to demonstrate the druggability of the interaction to design and develop chemical inhibitors disrupting the interaction. This study of our imparts and supports the previous literature in the understanding of TPD52 functions in prostate cancer.