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TGF- β mediated DNA methylation in prostate cancer

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Abstract: Almost all tumors harbor a defective negative feedback loop of signaling by transforming growth factor- β (TGF- β). Epigenetic mechanisms of gene regulation, including DNA methylation, are fundamental to normal cellular function and also play a major role in carcinogenesis. Recent evidence demonstrated that TGF- β signaling mediates cancer development and progression. Many key events in TGF- β signaling in cancer included auto-induction of TGF- β 1 and increased expression of DNA methyltransferases (DNMTs), suggesting that DNA methylation plays a significant role in cancer development and progression. In this review, we performed an extensive survey of the literature linking TGF- β signaling to DNA methylation in prostate cancer. It appeared that almost all DNA methylated genes detected in prostate cancer are directly or indirectly related to TGF- β signaling. This knowledge has provided a basis for our future directions of prostate cancer research and strategies for prevention and therapy for prostate cancer.

Keywords: TGF- β ; DNA methylation; prostate cancer; DNMT; Erk activation; tumor development and progression



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Introduction

The underlying mechanism promoting tumor progression has been elusive. Almost all tumors harbor a defective negative feedback loop of signaling by transforming growth factor- β (TGF- β). TGF- β signaling consists of Smad and non-Smad pathways (1). In advanced cancer cells, the non-Smad pathways predominate and progress leading to deregulated signaling cascades (2). This deregulation creates a unique TGF- β mediated tumor microenvironment that sets off a vicious cycle and promotes many of the hallmarks of tumor progression, including sustained angiogenesis, immune system evasion, proliferation, loss of the apoptotic response, epithelial-to-mesenchymal transition (EMT) and metastasis. These combined effects lead to uncontrolled

tumor growth and spread, for which we coin the term "TGF- β mediated vicious cycle in tumor progression". Recent evidence demonstrated that TGF- β mediates aggressive cancer including auto-induction of TGF- β 1 and increased expression of DNA methyltransferases (DNMTs) (2,3). This latter observation suggests that the expression of these methylated genes may be an important event in TGF- β mediated tumor progression.

DNA methylation in cancer

Epigenetic changes are characteristic of nearly all malignancies and include changes in DNA methylation, histone modification and altered expression of microRNAs. DNA methylation plays a critical role in cancer development

and progression. Alteration of DNA methylation patterns leads to deregulation of gene expression, in the absence of mutation. In the past few years, there has been an explosion in the number of publications in DNA methylation in all types of cancers (900 papers as of March 2012), including representative publications in prostate cancer (4-7), bladder cancer (8), renal cell carcinoma (9), breast cancer (10), lung cancer (11), ovarian cancer (12), oral cancer (13), pancreatic cancer (14), and other cancers. All tumors that have been examined show changes in DNA methylation, suggesting that this may represent a basic element of cancer biology, which has a significant impact on tumor pathology. Readers are referred to many excellent reviews on the biology of DNA methylation (15-17). This increased interest in the study of DNA methylation has created an opportunity for us to query the relationship between TGF- β signaling and DNA methylation in cancer, which has not been appreciated to date.

Biology of TGF- β signaling

TGF- β is a potent pleiotropic cytokine that regulates mammalian development, differentiation, and homeostasis in essentially all cell types and tissues. Its signaling is mediated through Smad and non-Smad pathways to regulate transcription, translation, microRNA biogenesis, protein synthesis and post-translational modifications (1,18,19). TGF- β binds to the type II TGF- β receptor (T β RII) which recruits and transphosphorylates the type I TGF- β receptor (T β RI) (20). The activated T β RI then phosphorylates Smad2 and Smad3 at the c-terminus. Activated Smad2/3 forms heterooligomers with Smad4 and migrates to the nucleus to regulate transcription. The Smad complexes interact with a myriad of transcriptional co-regulators and other factors to mediate target gene expression or repression (21,22). Smad2/3 also interacts with and regulates microRNA processing. TGF- β also signals through a number of non-Smad pathways, including m-TOR, RhoA, Ras, MAPK, PI3K/AKT, PP2A/p70s6K, and JNK (1,23,24). Finally, a direct action of the activated T β RI can interact with eEF1A1 to block protein synthesis (19). Dysregulation of both Smad and non-Smad pathways is implicated in aberrant TGF- β signaling and its pro-tumorigenic events in advanced cancer (3).

TGF- β signaling and DNA methylation

TGF- β is a key regulator for DNA methylation through an increase in DNMTs expression, especially in cancer (3,12).

There exists a differential effect of TGF- β mediated DNMT activities between benign and malignant cells. In benign cells, TGF- β inhibits DNMT expression (25,26). In cancer cells, TGF- β stimulates DNMT expression (3,12). It should be noted that, in light of the importance of both TGF- β signaling and DNA methylation in tumor progression, the majority of the methylated genes in cancer are relevant to TGF- β signaling (12). This is consistent with our observation that over-expression of TGF- β and/or DNMTs is associated with aggressiveness and poor prognosis in prostate cancer (3,27).

Review of literature

In this review, we will focus our discussion in prostate cancer as an example, because the pattern of DNA methylation is organ specific. We surveyed the recent literature to identify the existing methylated genes in prostate cancer and attempt to determine which ones are mediated by TGF- β signaling. We have identified over 80 genes in which promoters are methylated in prostate cancer. This is a significant increase from 2006, when only 30 genes had been identified (28). Interestingly, the non-Smad pathways of known relevance to TGF- β are more often associated with de novo gene methylation (3,29). In contrast, the Smad-mediated pathways often lead to promoter de-methylation of genes (see below). In *Table 1*, we summarize the known TGF- β relevant genes in which the promoter becomes methylated in prostate cancer. We also identified those which have been known to be induced by TGF- β . Since, in advanced cancer cells, TGF- β induces the activation of Erk, JNK, AKT, and NF- κ B (1,3), the above methylated gene have been documented in the literature to be related with one of the above transcription factors, thus are considered as TGF- β relevant.

In addition, there are a few genes that are de-methylated and are mediated through Smad2/3 activation, such as α 2 [1] collagen (113), CD133 (26), and maspin (or SFN, 14-3-3 sigma) (41,59,67,114,115). However, a reversal of the methylation status in these genes can be observed in cancer cells when the TGF- β signaling events switched from the Smad pathways to the non-Smad pathways in cancer cells as in the case for maspin (116) and CD133 (117).

Table 2 lists genes that are not currently documented in the literature as TGF- β relevant. However, TGF- β mediates an over-expression of DNMTs in cancer cells, which is responsible for promoter methylation of these genes and. in non-cancer cells, TGF- β down-regulates the expression of DNMTs (25,26).

Table 1 Genes with known association with TGF- β that have DNA hypermethylation in prostate cancer

| Name | Function | Reference |
|-----------------------------|-------------------------------------------------|--------------|
| 1. TBRI | TGF- β receptor type I | (30,31) |
| 2. TBRIITGF- β | TGF- β receptor type II | (31,32) |
| 3. cdh13herin | Adhesion molecule, tumor suppressor | (33,34) |
| 4. TTP (tristetrapolin) | Loss of TTP stabilizes c-Myc mRNA | (35) |
| 5. TGFBI (Betaig-h3) | TGF- β induced gene | (36-38) |
| 6. IGFBP3 | IGF binding protein 3 | (39,40) |
| 7. beta 4-integrin | Promotes focal adhesion | (34) |
| 8. MAL | Promotes cell differentiation | (41,42) |
| 9. SLIT2 | Negative regulation of migration | (36,41,43) |
| 10. Bcl2 | Involved in apoptosis | (40,41) |
| 11. Caspase 8 | Pro-apoptotic gene | (44) |
| 12. EPHA7 | Tumor suppressor in prostate cancer | (45-47) |
| 13. BTG3 | Tumor suppressor | (48,49) |
| 14. PTGS2 | Pro-inflammatory enzyme | (50-52) |
| 15. HIN1 (or SCGB3A1) | Tumor suppressor | (41,53) |
| 16. RASSF1A | Tumor suppressor gene | (54-56) |
| 17. CHD13 | Adhesion molecule | (41,57,58) |
| 18. p15, p16, p21, p27, p57 | Cell cycle regulators | (57,59-61) |
| 19. RASSF1A | Pro-apoptotic, negative Ras effector | (41,62) |
| 20. TWIST1 | Suppressor of E-cadherin | (41) |
| 21. FHIT | Induces apoptosis through Bak | (63,64) |
| 22. SOCS3 | Negative regulator of cytokine | (65,66) |
| 23. TIMP-2, TIMP-3 | Inhibitors of metalloproteinase | (67-69) |
| 24. PITX2 | Activator of cyclin D2 | (41,70-72) |
| 25. DcR1, DcR2 | Fail to induced apoptosis through TRAIL | (73,74) |
| 26. GLIPR1 (or RTVP-1) | p53 target gene | (75,76) |
| 27. MGMT | DNA repair gene | (77-81) |
| 28. DKK3 (SFRP1) | Wnt antagonist | (82,83) |
| 29. RUNX3 | Tumor suppressor | (84-86) |
| 30. CAV-1 | Tumor suppressor | (87,88) |
| 31. Clusterin | Apoptotic protein | (89-91) |
| 32. TFPI2 (PP5, MSP1) | A potent inhibitor of matrix-metalloproteinases | (92,93) |
| 33. SOX7 | Suppressor of β -catenin | (94,95) |
| 34. SLC5A8 | Tumor suppressor | (96,97) |
| 35. SLC18A2 (or VMAT2) | Affects apoptosis and migration | (98,99) |
| 36. LPL | Tumor suppressor gene | (100,101) |
| 37. HRK (or ATF-2) | Proapoptosis | (102,103) |
| 38. INHBB | Inhibin betaB | (104,105) |
| 39. ID4 | Inhibitor of DNA binding | (41,106-108) |
| 40. FYN | Promotes proliferation and motility | (109,110) |
| 41. HPP1 (TMEFF2) | TGF- β signal pathway | (73,84) |
| 42. RRAD | Ras-related GTPases | (111,112) |
| 43. DRM/Gremlin | Down-regulated in Mos-transformed cells | (73,84) |

Table 2 Methylated genes in prostate cancer whose regulation by TGF- β is not yet known

| Name | Function | Reference |
|-------------------------------------|------------------------------------------------------|---------------|
| 1. HLAa | HLA class-I antigen | (41) |
| 2. ER β | Estrogen receptor | (67) |
| 3. ER α | Estrogen receptor | (67) |
| 4. AR | Androgen receptor | (67) |
| 5. RAR β | Tumor suppressor | (67) |
| 6. DAPK1 | Regulate cell death | (118) |
| 7. MDR1 | Multi-drug resistant gene | (41,119) |
| 8. APC | Antagonist of Wnt | (41,119-121) |
| 9. CD44 | Cell migration and adhesion | (52,57) |
| 10. MCAM (MUC18, CD146) | In advanced PCa | (41,122) |
| 11. TIG1 | Retinoic acid receptor responder | (41,123) |
| 12. THRB | Thyroid hormone receptor B | (41) |
| 13. Laminin-5 | Role in adhesion and motility | (124) |
| 14. WIF1 | Wnt inhibitory factor | (125-127) |
| 15. TSLC1 | Tumor suppressor | (128) |
| 16. RIZ1 | Rb-interacting zinc finger gene 1 | (73,129) |
| 17. Cyclin D2 (or CCND2) | Regulate cell cycle | (54,67,130) |
| 18. GSTP1 | Cell detoxification | (4,7,121,131) |
| 19. PDLIM4 | Actin binding protein, tumor suppressor | (41,132) |
| 20. Sprouty1 | negative regulators of MAPK/PI3K | (133) |
| 21. ZNF331 | Tumor suppressor | (134) |
| 22. TMS1(ASC, PYCARD) | Induces apoptosis by caspase | (57,73,135) |
| 23. GPX3 | Anti-oxidant | (82,119) |
| 24. NKX2.5 | Repress calreticulin expression | (41) |
| 25. NKX3.1 | Promotes normal differentiation | (136) |
| 26. DPYS | Sensitivity to 5-FU | (41,137) |
| 27. ENDRB | Endothelin receptor type B | (5,41) |
| 28. CADM2 | Cell adhesion molecule | (138) |
| 29. XAF1 | Interference with caspase inhibition of XIAP | (139-141) |
| 30. CRBP1 | Cellular retinol binding protein, promotes apoptosis | (73,142) |
| 31. FAS (TNFRSF6, APT1, CD95/Apo-1) | Induces apoptosis | (143) |
| 32. RPRM | Inhibits Cdc2-cyclin b1 activity | (73,123) |
| 33. GSTM1 | Detoxification | (82) |
| 34. EPB41L3 | Erythrocyte membrane protein band 4.1-like 3 | (28) |
| 35. SCTR | Gene encoding the secretin receptor | (105) |
| 36. SOCS1 | Negative regulator of cytokine | (73,84) |
| 37. HIC | Tumor suppressor | (79,81) |

DNA methylation associated with tumor initiation and progression

A characteristic of DNA methylation in cancer is its heterogeneity. Despite of this variation, some trends can

be discerned. We rationalize that genes that are widely methylated are likely involved during early stages of tumor development, such as GSTP-1 (4), which may be used for the early detection of prostate cancer. Many investigators

have used specific methylation pattern for prediction of cancer progression. However, during progression of prostate cancer, the tumor becomes increasingly heterogeneous, it will be difficult to pinpoint which genes are methylated that can be used as a prognostic marker and such efforts have been met with mixed results (144). It is reasonable to assume that as tumors progress, there will be more genes that undergo promoter methylation and demethylation. Therefore, the development of a rapid analysis of DNA methylation profile make it possible to follow the methylation patterns which may be used as an indication of disease progression.

Conclusions and future directions

Based on the present review, it is apparent that TGF- β signaling and DNA methylation are two important events in prostate cancer development and progression. In tumor progression, the deregulated TGF- β signaling mediates an increase in the number of genes undergoing DNA hypermethylation. These genes are generally associated with prevention of apoptosis, promotion of proliferation, facilitation of cell migration and evasion of the immune surveillance, resulting in tumor progression. In the era of personalized medicine, it becomes more important that we clearly define which genes are affected by TGF- β signaling and which genes are promoter hypermethylated during prostate cancer progression. Recent reports point out that some dietary and lifestyle interventions in cancer patients are mainly mediated through a reduction in DNA methylation (125,145,146), while others may lead to both gains and losses (147). It is possible that these dietary and lifestyle factors may be mediated at least partly through a normalization of the vicious cycle of TGF- β signaling in cancer microenvironment (148).

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Footnote

Conflicts of Interest: M. McClelland and D. Mercola are cofounders Proveri Inc., which is engaged in translational

development of aspects of the subject matter. The other authors have no conflicts of interest to declare.

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