Review Article

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-β1 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

Submitted Apr 15, 2012. Accepted for publication May 04, 2012.
doi: 10.3978/j.issn.2223-4683.2012.05.06
Scan to your mobile device or view this article at: http://www.amepc.org/tau/article/view/692/785

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-β1 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 (TβRII) which recruits and transphosphorylates the type I TGF-β receptor (TβRI) (20). The activated TβRI 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-Samd pathways, including m-TOR, RhoA, Ras, MAPK, PI3K/AKT, PP2A/p70s6K, and JNK (1,23,24). Finally, a direct action of the activated TβRI 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-κB (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 α2 [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 though 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, MSPI)	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. ERβ	Estrogen receptor	(67)
3. ERα	Estrogen reeptor	(67)
4. AR	Androgen receptor	(67)
5. RARβ	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 wildly 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).

Acknowledgements

Funding: Studies in this report are supported by research grants from the National Cancer Institute (U01 CA152738 EDRN, UO1 CA114810 SPECS, P50 CA90386 SPORE) and the Department of Defense (W81XWH-09-1-0311 and W81XWH-08-1-0720).

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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Cite this article as: Lee C, Zhang Q, Zi X, Dash A, Soares MB, Rahmatpanah F, Jia Z, McClelland M, Mercola D. TGF-β mediated DNA methylation in prostate cancer. Transl Androl Urol 2012;1(2):78-88. doi: 10.3978/j.issn.2223-4683.2012.05.06

