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RESEARCH HIGHLIGHT

A tight junction between E-Cadherin and the prostate tumor suppressor SPDEF

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Mounting evidence is highlighting the role of the ETS factor SPDEF as a prostate tumor suppressor. In a recent paper by Pal *et al.*, SPDEF was found to transcriptionally control the expression of E-Cadherin, an adhesion molecule with a crucial role in preventing metastatic spread.

Prostate cancer is the second leading cause of cancer death in men. Thanks to the advent of prostate-specific antigen testing in the 1980s, many patients now present with the earliest and most curable form of the disease. Despite this fact, a non-negligible fraction of them experiences metastatic recurrence, which is ultimately responsible for patients' death. The recent approval of new agents including abiraterone acetate, cabazitaxel, sipuleucel-T and denosumab has significantly enriched the therapeutic opportunities for advanced prostate cancer.[1] However, in order to improve future drug development and patient management, a deeper elucidation of the mechanisms of metastasis formation is of utmost importance, and surrogate biomarkers able to distinguish aggressive from indolent cancers are urgently needed.

The prostate-derived ETS factor SPDEF (alias PDEF or hPSE) is the latest discovered member of the ETS family, a large family of 27 transcription factors sharing a conserved DNA-binding domain and involved in normal and pathological processes by activation or repression of a multitude of target genes.[2] ETS family members have major roles in tumorigenesis, especially in prostate cancer where some of them like ERG, ETV1 and ETV4 are involved in recurrent chromosomal translocations with the prostate-specific, androgen-responsive protease TMPRSS2.[3]

Originally described as a prostate specific mRNA transcript, SPDEF expression is actually highly restricted to epithelial cells and has been found also in breast, colon, ovary, gastric, and airway epithelium. SPDEF loss during tumor progression has been documented in prostate, breast, ovarian and colon cancer.[4] Very recently, analysis of SPDEF levels in more than 500 human colorectal cancer samples demonstrated loss of SPDEF in approximately 85% of tumors, with a correlation with cancer progression.[5] Likewise, immunohistochemical analysis of SPDEF expression in prostate carcinomas demonstrated that highly invasive and aggressive tumors (high Gleason score or tumor stage) have low to no detectable levels of SPDEF. Most importantly, patients with SPDEF-positive tumor survived significantly longer than patients with SPDEF-negative tumor, with an 8-year survival rate of 94% and 40%, respectively.[6]

The role of tumor and metastasis suppressor of SPDEF is thought to be due to deregulation of a plethora of target genes. Among others, SPDEF has been reported to activate the transcription of the cell cycle inhibitor p21 and to repress several genes including the apoptosis inhibitor Survivin, the matrix degrading metalloproteinase MMP7, MMP9 and MMP13, the antimetastatic protein Maspin1 and the regulator of the epithelial–mesenchymal transition Slug.[4]

Epithelial–mesenchymal transition is a transdifferentiation process by which cancer cells lose cell polarity and junctions, detach from epithelial sheets and acquire the capability to move to distant sites by reprogramming to a fibroblast-like state.[7] A major hallmark of this process is the loss of E-Cadherin, a calcium-dependent adhesion molecule that is highly expressed in normal epithelial cells and well-differentiated tumor cells, while largely reduced in undifferentiated cancers.[8] Loss of E-Cadherin expression has been regarded as a central event in the switch to epithelial–mesenchymal transition and tumor metastasis and is regulated at both the genetic and epigenetic level. Several proteins such as SNAI1/SNAIL, ZFHX1B/SIP1, SNAI2/SLUG, TWIST1 and DeltaEF1 have been found to repress E-Cadherin expression.[9]

In a recent paper published in *The Journal of Biological Chemistry*, Pal *et al.*[10] contributed to unravel the anti-metastatic function of SPDEF by discovering a direct link between SPDEF and E-Cadherin transcription. The analysis of SPDEF and E-Cadherin levels in normal immortalized prostate epithelial cells, RWPE-1, and in a panel of four prostate cancer cell lines highlighted a direct correlation between the levels of the two molecules, and an inverse correlation with the tumorigenic and metastatic potential of cancer cells. The authors then demonstrated that stable overexpression of SPDEF in aggressive PC3 prostate cancer cells was able to increase E-Cadherin expression, while shRNA-mediated SPDEF knockdown in less aggressive LNCaP cells resulted in significant E-Cadherin decrease. Interestingly, complementary experiments with overexpression and interference of E-Cadherin ruled out a reciprocal influence on SPDEF expression. An even tighter link between SPDEF and E-Cadherin was documented by the identification of six ETS putative binding sites on E-Cadherin promoter. Chromatin immunoprecipitation and luciferase assays experimentally validated the transcriptional induction of E-Cadherin by SPDEF. Further functional experiments confirmed a reduction of migration and invasion upon overexpression of SPDEF and E-Cadherin in PC3 cells and an increase upon SPDEF knockdown in LNCaP cells. Finally, interference of E-Cadherin in cells with higher or ectopic SPDEF expression was able to rescue the impaired migration and invasion.

All together, the paper strengthens the emerging role of SPDEF as a metastasis suppressor and prognostic indicator of aggressive prostate cancer by adding the

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master molecule E-Cadherin to the list of its target genes. It is not stated whether the formerly identified SPDEF target Slug, which is known to repress E-Cadherin transcription, participates in the modulation of the protein in this system. The results are in agreement with previous findings that reintroduction of SPDEF in highly migratory and invasive PC3 cells results in re-establishment of cell polarity and formation of spheroid acinar-like structures in three-dimensional cultures in Matrigel.[11] Reduction of MMP9 upon SPDEF overexpression is also considered to contribute to this phenotype, thus highlighting the central role of SPDEF in the control of a network of genes involved in the metastatic process. Consistently, it has recently been demonstrated also in metastasis mouse models that SPDEF can diminish the ability of disseminated tumor cells to survive at secondary sites and establish micrometastases.[12]

The compelling evidence of a major role of SPDEF loss in prostate cancer progression warrants further research on this molecule, especially to deepen in further *in vivo* models the anti-metastatic properties of SPDEF. Also, it would be of interest to extend to other tumor entities the involvement of SPDEF in E-Cadherin transcriptional regulation and, more in general, the consequences of its loss in the process of metastasis formation. In this respect studies on SPDEF knockout mice, which were reported to be threefold more sensitive to develop colon cancer when properly crossed or treated,[5] will provide valuable information on the role of SPDEF in cancer initiation and progression.

A further open question concerns the mechanisms underlying SPDEF reduction. A detailed study of its promoter is currently underway and will certainly shed light on this issue, possibly opening new perspectives for SPDEF reconstitution. Interestingly, re-expression of SPDEF has been reported to be induced by methylseleninic acid (MeSA), a selenium-based compound that revealed potent anticancer activity *in vitro* and in preclinical models.[13] It has also been reported that SPDEF mRNA and protein levels often do not correlate,[14] suggesting a post-transcriptional control of SPDEF protein in carcinoma cells. Consistently, repression of SPDEF translation by two microRNAs, miR-204 and miR-510, has been documented.[15] A widespread upregulation of these microRNAs throughout prostate cancer progression remains to be determined and may eventually pave the way towards the identification of new biomarkers of tumor aggressiveness and/or anti-microRNA based therapeutic opportunities.

1 Dayyani F, Gallick GE, Logothetis CJ, Corn PG. Novel therapies for metastatic castrate-resistant prostate cancer. *J Natl Cancer Inst* 2011; **103**: 1665–75.
2 Sharrocks AD. The ETS-domain transcription factor family. *Nat Rev Mol Cell Biol* 2001; **2**: 827–37.
3 Tomlins SA, Rhodes DR, Perner S, Dhanasekaran SM, Mehra R *et al*. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. *Science* 2005; **310**: 644–8.
4 Steffan JJ, Koul HK. Prostate derived ETS factor (PDEF): a putative tumor metastasis suppressor. *Cancer Lett* 2011; **310**: 109–17.
5 Noah TK, Lo YH, Price A, Chen G, King E *et al*. SPDEF functions as a colorectal tumor suppressor by inhibiting β-catenin activity. *Gastroenterology* 2013; **144**: 1012–26.
6 Ghadersohi A, Sharma S, Zhang S, Azrak RG, Wilding GE *et al*. Prostate-derived Ets transcription factor (PDEF) is a potential prognostic marker in patients with prostate cancer. *Prostate* 2011; **71**: 1178–88.
7 de Craene B, Berx G. Regulatory networks defining EMT during cancer initiation and progression. *Nat Rev Cancer* 2013; **13**: 97–110.
8 Frixen UH, Behrens J, Sachs M, Eberle G, Voss B *et al*. E-Cadherin-mediated cell–cell adhesion prevents invasiveness of human carcinoma cells. *J Cell Biol* 1991; **113**: 173–85.
9 Peinado H, Olmeda D, Cano A. Snail, Zeb and bHLH factors in tumour progression: an alliance against the epithelial phenotype? *Nat Rev Cancer* 2007; **7**: 415–28.
10 Pal M, Koul S, Koul HK. The transcription factor SPDEF is required for E-Cadherin expression in prostate cancer cells. *J Biol Chem* 2013; **288**: 12222–31.
11 Johnson T, Koul S, Kumar B, Khandrika L, Venezia S *et al*. Loss of PDEF, a prostate-derived ETS factor is associated with aggressive phenotype of prostate cancer: Regulationof MMP 9 by PDEF. *Mol Cancer* 2010; **9**: 148.
12 Steffan JJ, Koul S, Meacham RB, Koul HK. The transcription factor SPDEF suppresses prostate tumor metastasis. *J Biol Chem* 2012; **287**: 29968–78.
13 Zhao H, Whitfield ML, Xu T, Botstein D, Brooks JD. Diverse effects of methylseleninic acid on the transcriptional program of human prostate cancer cells. *Mol Biol Cell* 2004; **15**: 506–19.
14 Nozawa M, Yomogida K, Kanno N, Nonomura N, Miki T *et al*. Prostate-specific transcription factor hPSE is translated only in normal prostate epithelial cells. *Cancer Res* 2000; **60**: 1348–52.
15 Turner DP, Findlay VJ, Moussa O, Semenchenko VI, Watson PM *et al*. Mechanisms and functional consequences of PDEF protein expression loss during prostate cancer progression. *Prostate* 2011; **71**: 1723–35.