The EZH2 polycomb transcriptional repressor—a marker or mover of metastatic prostate cancer?

The recent finding of overexpression of the polycomb group transcriptional repressor EZH2 in prostate cancer raises the possibility that transcriptional regulation at the chromatin level may play a role in the development of the metastatic phenotype and suggests new avenues of exploration with respect to patient stratification and therapeutics.

The treatment of prostate cancer patients is plagued by the heterogeneous clinical behavior of cancers ranging from indolent tumors requiring no intervention to aggressive tumors resulting in patient mortality. Exemplary of the difficulties arising from such heterogeneity are the results of the randomized trial of surgery versus observation (Holmberg et al., 2002). In this study, where surgery was associated with a 50% reduction in hazard ratio of death from prostate cancer, the absolute reduction in mortality was a modest 6.6% suggesting that the benefit of surgery was confined to a subpopulation of men. Thus, there is a critical need to define unique prostate cancer subgroups differing with respect to clinical outcome.

A number of clinical and pathological features including the preoperative prostate-specific antigen (PSA) level, the clinical stage, and the state of tumor differentiation (Gleason Score), can stratify patients into subgroups differing with respect to outcome after surgery and are widely used to guide clinical decision making. In the era of PSA-based cancer detection, patients are increasingly presenting within a narrow range of these parameters and as such these measures are beginning to lose their discriminatory power.

Molecular approaches to this problem have focused on candidate gene analysis in tissue-based assays. The loss of expression of genes with tumor suppressor activities including p27, PTEN, and E-cadherin has been associated with disease progression or with higher grade tumors, while overexpression or amplification of putative oncogenes including Her-2/neu, Bcl-2, myc, and cyclin D1 has been noted in aggressive androgen-independent disease (reviewed in Thomas and Loda, 2002). However, none has gained widespread use in clinical practice.

The lack of progress in defining useful molecular markers and in understanding the deregulated biological processes driving prostate cancer cell transformation has led to the application of genomic approaches to this problem. Microarray expression experiments have identified a number of additional molecular markers including hepsin, AMACR, PIM-1 kinase (Dhanasekaran et al., 2001; Magee et al., 2001; Welsh et al., 2001), or expression-based models as potential indicators of disease or predictors of disease progression (Singh et al., 2002).

Varambally et al. (2002) using cDNA microarray analysis compared the gene expression patterns found in benign prostate tissue, organ-confined tumors, and androgen-independent metastatic prostate tumors—the penultimate lethal form of the disease. Here, they found that the polycomb group (PcG) protein enhancer of zeste homolog (EZH2) was among a group of transcripts whose increased expression distinguished metastatic tumors from those localized to the prostate. The authors show that the expression of both the EZH2 mRNA and protein progressively increase from benign to organ-confined, to metastatic tumors, suggesting that increases in EZH2 precede the development of metastatic foci. These data raise the possibility that EZH2 protein levels might prove useful in predicting patient outcome after prostatectomy. Indeed, immunohistochemical analysis of the EZH2 protein in tissue microarrays predicted outcome independently of Gleason Score, presurgical PSA, and stage. If validated in larger datasets these data could provide important additional information useful in patient stratification.

During development PcG proteins are required to maintain the appropriate silencing or repression of homeotic genes through the recruitment of chromatin modifiers to Polycomb response elements and are opposed in action by the trithorax group of proteins (TrxG). More specifically while TrxG proteins maintain the activated state of Hox genes in the appropriate developmental segments, PcG genes generate heritable states of gene silencing over extended regions of chromatin to prevent inappropriate Hox gene expression.

The protein products of the PcG family form two distinct complexes one containing EZH2/EED and a second HPC/HPH complex containing BMI1, an oncogene that cooperates with myc in lymphomagenesis and is amplified in mantle cell lymphoma. While gene amplification or direct transformation by members of EZH2/EED complex have not yet been demonstrated, EZH2 overexpression has been observed in lymphoma (reviewed in Jacobs and van Lohuizen, 2002). Moreover, EZH2 overexpression has been linked to increases in proliferation (Visser et al., 2001) whereas antisense inhibition of EZH2 has been associated with decreases in DNA synthesis (Fukuyama et al., 2000). Similarly, in prostate cells Varambally et al. show that RNAi-mediated EZH2 knockdown leads to growth inhibition in cell culture and that this may be linked to alterations in the cell-cycle profile. These data suggest, but do not constitute definitive evidence of a link between EZH2 activity and either the process of cellular transformation or the induction of a metastatic phenotype. Thus, a necessary step is to determine whether EZH2 has transforming activity or can confer metastatic potential. In addition, evidence that EZH2 is targeted by specific genetic alterations, such as gene amplification, would likewise bolster the argument that EZH2 expression is functionally relevant in prostate cancer.

The molecular mechanisms that might link PcG proteins such as BMI1 and EZH2 or TrxG proteins to the process of transformation remain unclear. EZH2/EED clearly functions as a transcriptional repressor complex and may do so by recruiting histone deacetylase or histone methyltransferase activities to chromatin (Cao et al., 2002; van der Vlag and Otte, 1999). TrxG complexes are associated with transcriptional activation, and in human leukemia the TrxG gene *MLL* is a major target of translocation. However, in some cases such transloca-

tions create MLL-fusion proteins that recruit PcG repressors to promoters (reviewed in Jacobs and van Lohuizen, 2002). These data suggest the intriguing possibility that overexpression or amplification or PcG proteins such as BMI1 and EZH2 participate in transformation through their native transcriptional repression activities while the TrxG protein MLL may transform through the acquisition of PcG-like repressor activities.

In leukemia a number of translocations often involving transcription factors, are recognized as having created novel fusion proteins strongly linked to the development of the disease. Notable among these are the AML1-ETO and PML-RARα fusions which generate unique transcriptional repressor proteins. Moreover, in mouse models of leukemia recent data suggest the possible utility of treating this disease by reversing transcriptional repression with histone deacetylase inhibitors (He et al., 2001). These data have led to the initiation of clinical trials in selected leukemia patients. The implication that aberrant EZH2 transcriptional repression mediated through histone deacetylase may contribute to or drive aggressive forms of prostate cancer, raises the possibility that novel "transcription"-based therapeutics might find clinical utility in the treatment of this disease.

If transcriptional repression by EZH2 seems a likely molecular mechanism through which growth advantage is obtained, what are the genes targeted for repression? Here, Varambally et al. use transcriptional profiling in cell culture systems to identify a number of putative EZH2 repressed transcripts. However, surprisingly the authors did not show whether such targets were corepressed in the expression profiles from the metastatic human prostate tumors. Among the downregulated genes were numerous solute transporters, but otherwise no strong functional themes emerged.

Based on the currently available data EZH2 transcriptional repression may be linked to a deregulation of cell proliferation. Nonetheless, an alternative model is worth considering. In acute leukemia, emerging data suggests that specific translocations, typically involving transcription factors, induce a block in myeloid differentiation and cooperate with those translocations triggering kinase activation to induce full-blown leukemia (Deguchi and Gilliland, 2002). In prostate cancer, alterations in differentiation are clearly evident and less well-differentiated tumors are associated with a poor outcome. To date, no mechanism accounting for this apparent block has been elucidated. Given the role of PcG proteins in blocking the differentiation program during development through gene repression, a possibility worth exploring is that EZH2 overexpression may act to promote prostate cancer development through the initiation of inappropriate block to prostate epithelial cell differentiation.

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Selected reading

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