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RESEARCH HIGHLIGHT

A fusion at the root of prostate cancer

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Many hematopoietic malignancies have oncogenic gene fusions, like BCR-ABL, in their tumor-initiating cells. This implicates the product of the fusion as a powerful cancer-initiating event. In human prostate cancers, despite the detection of numerous similar fusions, e.g., the *TMPRSS2-ERG* fusion, there was no evidence for its presence in the root of the cancer, the cancer stem cells. Polson *et al.* now not only report that the fusion is indeed present and active in tumor initiating cells from human prostate cancers, but that its expression is tightly controlled by epigenetic mechanisms, with potential impacts on tumor latency and treatment.

Prostate cancers are heterogeneous, not only in their appearance, but also in their responses to even the most sophisticated molecularly targeted treatments. By developing methods to fractionate, culture and study the individual cell types, which comprise a human prostate cancer, it may be possible to identify the optimal agent, or combination of agents to provide a longer-lasting treatment than those currently available, particularly for Castration resistant disease.

As a demonstration of the extra information to be gained by cellular fractionation, in our recent paper in *Nature Communications*,[1] we were able to demonstrate not only the presence, but also the expression of the cancer-associated *TMPRSS2-ERG* fusion in the stem cell fraction from human prostate cancers. The obvious conclusion is that *TMPRSS2-ERG* is a cancer-initiating mutation, which acts in stem cells. This is not without controversy, as the *TMPRSS2* promoter has been described as androgen regulated[2] and that the *TMPRSS2-ERG* fusion can be induced by androgen receptor action.[3] However the colony forming cells in our experiments did not express any detectable androgen, or indeed estrogen receptors, although we cannot exclude a gross de-differentiation of androgen receptor-expressing luminal cells into these primitive basal undifferentiated stem cells. The result does provide a stronger basis for the importance of *TMPRSS2-ERG* as an initiating mutation for some prostate cancers and for the stem cell hypothesis of prostate cancer.

There also remains some controversy as to whether expression of *TMPRSS2-ERG* predicts a poor outcome in prostate cancer. Our data show that when the fusion is present in stem cells, it is always expressed. This implies that it is important for cancer stem cell function or survival. However, with extended time in culture and clonal selection for stem cells, the *TMPRSS2-ERG* colony-founding cells were eventually eliminated. This agreed with older data[4] that such primary cultures, initiated from enriched stem cells, do change their nature and appearance with extended passage. There are two further interpretations of this result.

Firstly, culture medium almost certainly selects for specific cell types—analogous to selection by the microenvironment in a tissue (intraprostatic or metastatic site). It is perhaps notable that only VCaP of the established *in vitro* prostate cell lines contains and expresses the *TMPRSS2-ERG* fusion, despite the presence of the fusion in 40%–50% of all prostate cancers. By a selection mechanism, the fusion-containing cells are gradually competed out by faster growing cell types. *In vivo* it is clear that prostate cancers are of multi- or oligo- clonal origin[5] and develop/differentiate on a clonal basis, even after treatment, so there is the capacity for such microenvironmental (or indeed treatment induced) selection for a new clonal growth. There is convincing genetic evidence that secondary tumors are genetically related to the primary lesion,[6] but this does not occur in all cases. An alternative explanation is that we start our primary cultures with a mixture of normal (fusion-negative) and tumor cells, and that the *in vitro* conditions promote the growth of normal epithelium. For such studies on primary tumor material, it is essential to modify the techniques to answer the scientific question, rather than grow sufficient cells to carry out a technique!

The second conclusion in the paper emphasized the stem cell nature of the colony-forming cells we isolated, based on their expression of CD133, high levels of $\alpha 2\beta 1$ integrin and CD44 expression.[4] While *TMPRSS2-ERG* was actively transcribed in the stem cells, when the stem cells divided to give a transit amplifying cell and another stem cell—the process known as self-renewal, we found that the active fusion present in the stem cells was almost always switched off in the transit amplifying cells, and that the other (unfused) allele of *TMPRSS2* was switched on, i.e., a chromosomal example of asymmetric division and allelic exclusion, which is often seen in embryonal tumors such a Wilms tumor.[7]

So why is this interesting? Firstly, it can explain why certain activated genes are only expressed in a certain proportion of cells in a cancer (heterogeneity), which might explain why specific targeting of all cells in the cancer by a reagent against an activated oncogene has proved difficult. This is particularly true if the temporal activity of this oncogene (*ERG*) during cancer development (in pre-tumor progression in a small proportion of stem cells), and the cell type in which it is most active (activated stem cells) are not the luminal cell types being targeted by the therapy.

Secondly, the strength of the silencing and switching (which is epigenetic, but as we demonstrated in the paper was not due to genomic methylation of the *TMPRSS2* promoter) means that a potent cancer-inducing fusion like *TMPRSS2-ERG* could be completely

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silent in a proportion of cells in a cancer. If this silencing happened during the development of a cancer, then it provides a mechanism whereby such mutations can accumulate over time. Most premalignancies 'appear' with a set of mutations and the term pre-cancer progression has been used for the time period when such mutations are accumulating, without any obvious effects on, e.g., cell morphology. Given that mice induced to overexpress ERG, and containing PTEN deletions rapidly develop premalignant lesions into invasive cancer, then why are such early changes not seen in man?[8] Allelic silencing would provide just such a mechanism.

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