N-Myc-mediated epigenetic reprogramming in advanced prostate cancer: personalized medicine and quality of biological samples

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Prostate cancer (PCa) is a health issue, but also a great scientific challenge. The latency of PCa is very long. A population-based study in Sweden showed a PCA-specific death rate of 1% over the 10 years after diagnosis of low-risk PCA in men 70 years old at diagnosis, and lower rates for men younger at diagnosis (1,2). We do not understand why: the behavior of PCa and its progression are still difficult to predict. PCa is highly heterogeneous, both morphologically and from the molecular point of view. By a Darwinian, we must consider that the epithelial cells from which it originates undergo a constant selective pressure to adapt to the biological environment of the patient since its very beginning, more or less 15–20 years before PCa is diagnosed. The tumor continues to evolve a long time before PCa kills the patient. Cancer-originating cells evolve slowly, but inexorably, into clones that are increasingly suitable for making their way into the gland, disrupting its tissue organization, come out of it, and colonize other tissue, like the bones. Derangement of gene expression and changes in metabolic pathways (both faces of the same coin) clearly occur. Cancer can be seen as a progressive loss of order, i.e., a kind of "biological entropy". This is why time (aging) is the most important risk factor for PCa. A major challenge for the researcher lies in identifying the molecular determinants that may allow the early identification of aggressive disease. In addition, we need to discover new molecular targets, and novel therapeutic strategies. It is a typical translational research field, in which the new discoveries made *in vitro* in the laboratory must be rapidly challenged *in vivo*, and brought to the patient as soon as

possible. However, haste can be a bad advisor. Because of the urgent demand for breakthrough in this field, we may be pushed to announce important solutions too soon. Actually we must admit that the increase in overall life expectancy for aggressive PCa is still unsatisfactory after many years of research.

Dr. Berger and Collaborators published a paper (3) in which N-Myc was suggested to reprogram PCa cells both epigenetically and transcriptionally, leading to the acquisition of an alternative lineage identity, which is androgen independent. N-MYC is a well-known oncogenic transcription factor, previously found overexpressed in several kinds of neurological cancers as well as non-neuronal tumors, thus strongly suspected of being one of the main culprit of cell transformation (4). Perhaps this is an obvious observation. But what about in the case of the PCa? Well, naïve PCa may evolve in a histological entity called neuroendocrine carcinoma (NEPC). Treatment options for NEPC are unsatisfactory, and prognosis is poor for the patient. The possibility that N-MYC could be a trigger of PCa progression and NEPC development was previously described (4,5) also by members of the same research team (6). Now, the Authors provide an overwhelming amount of experimental data to support the hypothesis that N-MYC may be responsible for all that. They also described a gene signature that may help to identify NEPC and to describe the evolution of PCa in NEPC.

The bulk of knowledge provided by the paper is important and may effectively stimulate new approaches and accomplishments in the field. However, why it is so

difficult to understand the molecular determinants of PCa progression? One of the reasons is certainly related to which cells are we working on, and which experimental models we choose to use to collect the data. PCa is a heterogeneous and elusive disease. Unfortunately, the experimental models available are far from being a realistic reproduction of the actual disease in the patient. Even in this noteworthy paper, the Authors had to deal with different models, from LNCaP cells to transgenic mice and up to tissue specimens from patients. A typical translational research approach. It is good news in science when a novel observation is confirmed throughout the different experimental models investigated. A possible risk is that we carry on the same artifact from one system to the other. Only time will tell, because science requires further confirmation, i.e., reproducibility.

Those who know about human tissue specimens are always concerned about which group of cells have been selected for the study. The features of the human specimens used for the study are not described in full detail. In addition, what about the "field-effect" (7,8)? Important molecular changes may occur in the cells surrounding the cancer cell nest. What is happening in cells, which are different from classical epithelial-derived cancer cells, such as cancer-associated fibroblasts (9)? It would be very important to know: which cells start the N-MYC reprogramming first?

Studies such as this involve a huge amount of work, very sophisticated methods, and extract an enormous amount of information even from a few cells. The most important concern for us should be the quality of the tissue specimen. The Best Practices for biospecimens from the NCI demand the use of the highest quality samples, based on the intended research use (10). The issue of the right tissue samples is fundamental for precision medicine as well, also considering the tremendous amount of data that we can actually retrieve from a single specimen. Precision medicine strictly depends on quality and feature of tissue samples. Sample inadequacy as a cause of assay failure, currently reported for 15–20% of patients when the remaining tissue block from a biopsy is the only source of analytes. Blind biopsies are reported to fail 10% of the time. Although analysis of mRNA extracted from FFPE samples has improved, this mRNA is still degraded. In an ideal scenario, one sample of fresh tissue, histologically well characterized, should be the best option. The increase in sensitivity of the methods and the need for smaller amounts of tissue for molecular analysis may make the use of this kind of samples possible, and actually sufficient, for all molecular tests contemplated in precision medicine. In addition, this approach would reduce between-sample variance.

Actually, I must recall that we spent many efforts on ensuring the quality of fresh human tissue samples since long time ago (11,12). We step-sectioned cancer and normal pieces from fresh-frozen prostates, used thin sections every 0.2 mm to sandwich the tumor, which was then dissected out. This careful approach allowed us to find novel insights about molecular changes during cell transformation, and to identify a gene signature for PCa diagnosis (13) and progression (14) that was also confirmed in a transgenic mouse model (15-17). Since this procedure is useless for diagnosis, we invented a biopsy needle that can solve the problem of getting human fresh tissue: the Twin Sample biopsy needle (18). This needle obtains two samples in one pass, one for pathology and one for molecular assays. The two samples share a surface, as the two halves of a cylinder of tissue about one mm across. The pathology report informs the molecular analysis, since the percentage of tumor cells, which affects the depth of coverage of sequencing. Because we need to know exactly which cells we are sequencing. The Twin Sample needle provides about 3 mg of fresh tissue, suitable for multiple molecular studies. This device hopefully will provide a new and handy tool to know exactly which cells we are working on. We foresee that the use of the Twin Sample needle will generate new and more reliable data on PCa and other diseases, and enable molecular diagnosis for patients.

In conclusion, the paper by Dr. Berger *et al.* is outstanding and deserves the praise of all. It tells us a rational and plausible scientific story based on the biology of N-MYC that suggest molecular targets, molecular determinants for patient classification, and possible therapies, which may make a difference in the future. Their next work will certainly tell us more. A novel pharmaceutical approach to cure aggressive PCa is still far from being discovered and novel drugs are still urgently needed.

But please do not forget that, when working on PCa, personalized medicine requires a perfect knowledge of the patient sample.

Footnote

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