

Driving in the melanoma landscape

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Abstract: The melanoma landscape is rapidly evolving. The melanoma oncologists have now the first successful targets for therapy that have a genetic base – albeit in rare forms of the malignancy. Once melanoma becomes part of the Cancer Genome Atlas consortium, a comprehensive map of genetic changes will be established to point the field to true drivers of the disease that will become new targets for therapy. The same abnormalities will

also serve as biomarkers for diagnosis, prognosis and therapy follow-up. Melanomas as a group are heterogenous as are tumor cells within one lesion. New strategies will move towards individualized therapies and combination therapies to target all cells within a tumor.

Key words: melanoma – stem cells – oncogenes – targeted therapy

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For long-term planning, in both government and industry, experts may perform a SWOT analysis, which stands for strength, weaknesses, opportunities and threats. Such analyses provide a valuable current-state assessment, on which one builds strategic plans for the future. Three years ago, a group of over 40 melanoma investigators developed a consensus 'Roadmap for Melanoma Research', which continues to guide our discussions (1). We started with a SWOT analysis of the current state of the melanoma landscape to better formulate what direction the field should move in. This initial analysis is outdated in the science areas but not with regard to the general needs of the field. Have we gotten stronger? The science has progressed significantly, however, lack of funding, particularly in the US, has prevented us from taking full advantage of this progress. Like in the previous decade, progress in basic science is outpacing that in the clinics and thus our challenge for the future is to better connect the laboratory with the clinics. Translational research is difficult to materialize, and the obstacles are formidable. We need to train a new generation of researchers who can seamlessly switch between clinical and basic research languages and who will drive the field by bringing the laboratory closer to the clinics. In this brief overview, we will focus mostly on the opportunities for the melanoma landscape and where we can expect the most progress in the next few years.

The drivers

Melanoma development, and likely also progression, depends on genetic alterations that drive the cells to grow, migrate, invade and survive in a 'hostile' world of hypoxia,

loss of attachment and cell death triggers both natural and induced by treatment regimens. Melanomas are 'addicted' to genetic oncogenes and tumor suppressor genes that are genetically altered. We can drive progression by stimulating the environment for the production of growth factors, but the lesions will collapse if there are no constitutive drivers. Which ones are important? Members of the MAPK pathway, the central proliferation pathway, are important, with most frequent mutations in BRAF, NRAS and c-kit. If we combine mutations in BRAF (50–60%), NRAS (15%) and c-kit (2–4%), we end up with approximately 70% of all cases that carry a mutation in one of these genes, which is a remarkably high frequency and apparently very important for the tumor. Because nevi already carry the mutations, at least for BRAF and also some for NRAS, we need to consider additional mutations that are drivers of melanoma. We can only speculate on the additional genes. Melanomas are very aneuploid and carry hundreds of mutations.

Unless we perform genome-wide sequencing, we will not find the correct answers on the hierarchy of gene mutations for a long time. Because melanomas are also very heterogeneous, we cannot get the answers from sequencing only a few specimens. The plans for such an undertaking are in place and the consensus among the experts of major sequencing centres is that 500 specimens are needed to cover all melanoma sub-types in sufficient numbers. What are the obstacles? At this time, most of the technologies are in place and it would take an estimated 5 years. What is the price tag? The costs for sequencing one genome are coming down rapidly. Two years ago, it was \$1–2 million, currently it is down 10 fold and it is expected that the price may decrease

to \$10 000/genome in a few years. Thus this project is not as unrealistic as it first seemed. Data on whole genome sequencing from three cancers are already becoming available giving us a first glimpse of the 'hills and mountains' of the cancer gene mutation landscape (2–4) but the funding agencies are not expected to open their purse for melanoma anytime soon. What can we expect? An abundance of 'noise', i.e. many genetic aberrations are secondary and not drivers due to the unstable genome. Distinguishing essential drivers from bystanders will require careful biological investigations. We expect dominating drivers and followers, where only the drivers will be of significance. The followers may have local importance but they will not influence tumor cells in major ways. Knowledge of the drivers will likely change current classification of melanomas, because risk assessment and treatment of melanoma will depend on who the driver is. For now, every melanoma patient receiving signal transduction inhibitors should be tested for major genes important for melanoma, and we include BRAF, NRAS, c-kit, CDK4, cyclin D1, p53, MDM2, PI3K α and AKT3 in our panel. The recently discovered gene GNAQ points to the importance of the PKC pathways in sub-groups of melanoma. For c-kit, cyclin D1 and CDK4 we should not only test for genetic abnormalities but expression changes as well. The first clinical successes have already been reported in a rare group of c-kit mutant melanomas (5,6) and tumors with overexpressed c-kit may also respond to c-kit antagonists like Gleevec[®] (7). Once we have the melanoma genome sequenced and have sorted through the forest of aberrations, we will go to the next frontier, epigenetic regulation of gene expression. Finally, we need to go to the 'business end' of genes, the proteins. microRNA investigations on gene regulation have exploded onto the science field, but at this time we can only speculate whether specific microRNA are critical for melanoma. This field is still evolving.

The cars

What once seemed clear to all of us, that one car, i.e. one cell fits all melanomas, is no longer as clear. Cutaneous melanomas were believed to be derived from cutaneous melanocytes. This is a generally accepted rule but it does not take into account that the skin contains stem cells and precursor cells, which can give rise to melanocytes, among many other cell types. These stem cells, which are found throughout the skin, including hair follicles and dermis, can be transformed at any given time during the melanocyte differentiation process because they respond differently to oncogenic stimuli and may not be driven into apoptosis as mature cells would. Thus oncogene-induced senescence may not hold up for stem cells.

There is another mechanism that may occur prior to or during transformation. A powerful reversal of the fate of cells

has recently been discovered in mouse and human fibroblasts and epithelial cells. Four genes, Sox2, Oct4, c-myc and Klf4 were required for transduction and then cells became embryonic stem-like cells. c-myc can be a powerful transforming gene for melanoma, and thus, attempts are ongoing to delete it. Instead of de-differentiation to embryonal stem cells, we are interested in induced melanocyte progenitor cells. Initial preliminary data are promising. Thus this field is very much in flux and the next few years will likely help us distinguish melanomas according to their cell of origin.

The roads

The size and shape of the roads depend on the landscape and on the need for traffic to move from point A to point B. The same holds for cancer cells. Tumor cells in an artificial environment, like the tissue culture dish, can manufacture all they need from the key ingredients in media, including amino acids, vitamins, salts and sugars, and we have grown melanoma cells for 2 years on such a minimal diet. In real life, tumor cells are embedded in a network of stromal cells, which together with the matrix represent the microenvironment.

In contrast to the normal cells, whose existence depends on a predetermined environment, tumor cells direct the stromal fibroblasts, endothelial cells and inflammatory cells to produce growth factors for them. Most importantly, they produce matrix proteins that the tumor cells use for adhesion and migration. A classical example is tenascin, which is not produced by most carcinoma cells but the tumor cells require it for migration. Instead of producing tenascin, the tumor cells produce a growth factor, TGF- β , which stimulates fibroblasts to produce tenascin (and other growth factors the tumor cells benefit from). Melanoma cells are special because they are not only well equipped to stimulate fibroblasts, endothelial cells, smooth muscle cells and inflammatory cells, but also have properties similar to such cells, which is mostly reflected by the molecules they express on their surface or secrete into their surroundings (including tenascin). Thus, melanoma cells have an endothelial, fibroblastic, or monocytic signature and they can have the same properties as the respective normal cells. This functional plasticity is a remarkable testament to the versatility of melanoma and a clear indicator of the difficulty in treating this tumor with conventional therapies. Studies of the tumor microenvironment are important but we have not yet determined how this increased knowledge can be translated to new advances in melanoma therapy or in the selection of patients for particular therapies.

The roadblocks

For the last three decades, we have placed one roadblock after another in front of melanoma cells and they have

circumnavigated them all with admirable elegance. Some treatments have slowed tumors down but all failed to significantly prolong survival. Thus none of the current therapies is good enough to kill all tumor cells. Once a small residual tumor is left, melanomas are expected to recur. Why can we not kill all tumor cells? We have clearly underestimated the ability of tumor cells to survive under the most difficult circumstances. We need road bombs instead of roadblocks to destroy all tumor cells. Let us learn from our 'mistakes', which are largely based on the scant knowledge of melanoma biology: (1) Not all melanomas are the same. We have treated all major melanoma forms as one disease expecting that all melanomas respond similarly to therapy. However, even in superficial spreading melanoma, we have several variants, mostly characterized by differences in oncogene and tumor suppressor drivers. There is no one-size-fits-all in this malignancy. Instead, there is increasing evidence that melanomas, more than other cancers, need individualized therapy. (2) Not All melanoma cells within one tumor are the same. Melanomas have often been described as heterogeneous based on cell shape and pigmentation. However, there is more to it. Tumor cells within one tumor respond differently to therapy because sub-populations have better efflux pumps for drug removal or they do not proliferate at all, making them inaccessible to proliferation antagonists that most of our drugs represent. (3) One bullet does not kill all. Melanomas have multiple pathways constitutively activated. Interfering with a given pathway/mechanism would not be sufficient to kill all tumor cells. We need to target the most critical pathways but do not yet know which are the most important. Only a small percentage of melanomas will show total dependence on one driver and will show response to one antagonist. The vast majority of melanomas will not. We need to develop combinations of drugs that have a strong rationale both for single agent therapy and for combination therapies.

The road ahead

Melanoma has defied all attempts for rapid cures. Clearly our past strategies with the heavy emphasis on immunotherapy have not provided the results we had hoped for. Is one therapy worse or better than the other? We cannot predict this and need to find out. We have to come to a consensus on certain issues: (1) Each therapeutic approach should have a strong rationale. For example, a specific antagonist for a signalling molecule should only be given to patients with a specific abnormality, not to those without it. Ideally, we should have strong preclinical data to

justify what we want to accomplish in patients. The melanoma models, particularly the genetic mouse models, are getting better and should be useful to develop a strong experimental foundation for therapy in patients. The increasing use of genetic mouse models that mimic the human disease and unravel the intricate signalling circuitries in melanoma cells help us to design new therapy strategies in patients. In addition, human melanoma cell lines are now available that represent each of the major mutational groups in melanoma. These cell lines will be made available to the melanoma research community (8) (2) There maybe exceptions in specific sub-groups of melanomas, but for the vast majority of melanomas we cannot expect that one drug kills all tumor cells. We need to use combination of drugs and those may vary between patients depending on the genetic signature of lesions (9). (3) Our goal should be cures. We may settle in some cases on stabilizing the disease but this should not be the ultimate goal. To achieve cures, we need to kill all tumor cells and not just most hoping for a bystander effect of tumor killing. Experimentally, nearly every melanoma cell isolated from patients' lesions can induce a tumor in immunodeficient mice (10). (4) Single laboratory efforts to develop new therapeutics are laudable but hardly efficient. We have to seek and develop new structures for collaborations. Of course, we can partner with industry but we cannot leave all the initiatives for the development of new therapeutics to industry. Academia has to jump in and make major commitments, particularly if the need for individualized therapy increases. We are 'forced' to work together in a close-knit network of collaborating laboratories that nevertheless maintain their independence.

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