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Review

Progress in understanding melanoma propagation

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ABSTRACT

Melanoma, like most cancers, is a disease that wreaks havoc mostly through its propensity to spread and establish secondary tumors at sites that are anatomically distant from the primary tumor. The consideration of models of cancer progression is therefore important to understand the essence of this disease. Previous work has suggested that melanoma may propagate according to a cancer stem cell (CSC) model in which rare tumorigenic and bulk non-tumorigenic cells are organized into stable hierarchies within tumors. However, recent studies using assays that are more permissive for revealing tumorigenic potential indicate that it will not be possible to cure patients by focusing research and therapy on rare populations of cells within melanoma tumors. Studies of the nature of tumorigenic melanoma cells reveal that these cells may gain a growth, metastasis and/or therapy resistance advantage by acquiring new genetic mutations and by reversible epigenetic mechanisms. In this light, efforts to link the phenotypes, genotypes and epigenotypes of melanoma cells with differences in their in vivo malignant potential provide the greatest hope of advancing the exciting progress finally being made against this disease.

1. Melanoma progression

Melanoma is eminently curable if primary tumors are detected at an early stage and surgically removed. Because of this, monitoring of high-risk patients and of pre-malignant lesions such as dysplastic nevi is frequently recommended. However, a high proportion of melanomas arise de novo and not in association with previously benign nevi (Weatherhead et al., 2007). Because of this, not all primary melanomas present at a stage that is reliably curable by surgery, and over 10% of patients present with metastatic disease (Hu et al., 2009). Therefore, despite the importance of understanding melanomagenesis to improve primary prevention, appreciating how melanomas propagate *after* the establishment of a primary

Abbreviations: CSC, Cancer Stem Cell; NSG, NOD/SCID IL2Rγ$^{-/-}$; NK, Natural Killer.

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tumor is critical to reduce the physical and economic burden of this disease.

Conceptually, cancer propagation is proposed to occur according to various models, each of which provides an independent explanation of the phenotypic and functional heterogeneity that is often apparent among cells within a malignant tumor. The first is the cancer stem cell (CSC) model (Dick, 2008; Lobo et al., 2007; Reya et al., 2001), in which tumor growth is primarily driven by rare populations of highly tumorigenic cells that not only renew their own malignant potential, but also give rise to bulk populations of other cells that are irreversibly less- and/or non-tumorigenic. Second is the clonal evolution model (Fearon and Vogelstein, 1990; Foulds, 1958; Lengauer et al., 1998; Nowell, 1976), in which a high proportion of cells in a cancer has the potential to drive disease progression and in which certain cells acquire additional genetic mutations that provide an advantage in growth and/or metastasis capability. More recently, the separate notion of cancer cell plasticity, or interconversion, has been increasingly recognized in the literature as contributing to cancer cell heterogeneity and progression of malignant disease (Gupta et al., 2009; Mani et al., 2008; Marusyk and Polyak, 2010; Pinner et al., 2009; Roesch et al., 2010; Sharma et al., 2010). The interconversion model refers to reversible switching of cancer cells between more and less actively malignant behaviors that may be associated with phenotypic distinctions and differences in therapy responsiveness between cells. In fact, although these models are conceptually quite different, they are not mutually exclusive, and it is likely that at least some cancers use more than one of these models at different stages, or even simultaneously, during their evolution in a patient (Marusyk and Polyak, 2010; Shackleton, 2010).

How does melanoma progress? From a clinical perspective, melanoma is generally considered to be a highly aggressive cancer, although a small subset of patients with metastatic melanoma has a relatively indolent disease course (Tsao et al., 2004). Histologically, mitoses are frequently apparent in sections of melanoma tumors and staining for proliferative markers such as Ki67 is usually positive (Ohsie et al., 2008). In this light, it would be surprising if melanoma progressed according to a model in which tumorigenic cells were rare. However, cellular heterogeneity is also a histological feature of many melanomas, and studies of cell surface marker expression indicate that multiple, phenotypically distinct subpopulations of melanoma cells exist within tumors (Fang et al., 2005; Quintana et al., 2008; Schatton et al., 2008).

The basis of this heterogeneity has been the subject of intense debate among melanoma biologists – and rightfully so. If melanoma cell heterogeneity develops in the context of a CSC model, then separate identification, study and therapeutic targeting of the rare tumorigenic cell population should result in great clinical benefit to patients. Furthermore, studying melanoma tumors as a whole is likely to mask the critical drivers of melanoma progression if these drivers are only present in a rare minority of the cells. However, if a high proportion of melanoma cells has tumorigenic potential and is subject to ongoing and extensive genetic and/or epigenetic change, the implications for managing this disease would be profound. For example, targeting single oncogenic mechanisms to which tumors are supposedly 'addicted' (Weinstein and Joe, 2008) may be only fleetingly beneficial in genetically unstable melanomas in which resistance mechanisms rapidly emerge. Similarly, cancer cells that are able to avoid therapeutic intervention by transiently switching to epigenetically-determined states of resistance may require a multipronged treatment approach (Sharma et al., 2010).

2. Melanoma and the cancer stem cell model

Several studies have correlated the phenotypic heterogeneity of melanoma cells with differences in cell behaviour. Fang et al. (2005) evaluated in vitro clonogenicity in melanoma cells by utilizing their ability to form spherical aggregates of cells in non-adherent culture conditions. Spherogenicity was identified in only a proportion of cells isolated from melanoma tumors, and at least some cells derived from spheres could form tumors when transplanted into immunocompromised mice. Heterogeneity of CD20 expression was noted among cells derived from melanoma sphere cultures, and cells from the $CD20^+$ subpopulation showed a greater capacity to form secondary spheres than $CD20^-$ cells, suggesting an association between marker expression and clonogenicity in cultured melanoma cells. In support of this concept, Gedye et al. (2009) identified subpopulations of $CD133^+$ cells in early passage melanoma cell lines that displayed increased clonogenicity in soft agar culture compared with $CD133^-$ cells. Interestingly, in this study no differences in two-dimensional adherent growth were seen between cells according to expression of CD133, indicating that culture conditions can affect the evaluation of clonogenicity in melanoma cells.

An important question arising from these studies is whether the cells that were not clonogenic in vitro were tumorigenic in vivo. Tumorigenicity, a key component of malignant behaviour, is a property of cancer cells that by definition can only be demonstrated in vivo. Although clonogenic potential must exist in a cell for tumor formation to occur, it is not known how well the normal in vivo environment that supports clonogenic tumor growth in patients is recapitulated in vitro. In fact, melanoma cells isolated from patients are often difficult to grow in culture, despite displaying overt tumorigenicity. We have tested melanoma cells purified from six tumors obtained from patients with metastatic disease for their ability to form colonies in culture conditions that we have found to be supportive of melanoma cell growth (Fig. 1a). Each melanoma contained a high proportion (13%–70%) of cells with tumorigenic potential when evaluated in immunocompromised NOD/SCID $IL2R\gamma^{-/-}$ (NSG) mice, a highly permissive model for this purpose (Quintana et al., 2008). However, only four of these melanomas contained cells that proliferated and formed colonies in non-adherent culture. In three experiments, we also cultured cells adherently after plating on tissue culture-treated plastic (adherent culture on Matrigel did not increase the detectable frequency of clonogenic cells compared to culture on plastic; data not shown). Although the frequency of detectable clonogenic cells was generally higher in adherent culture than in non-adherent culture (Fig. 1a), in most cases the proportion of colony-forming cells identified in any culture condition was several-fold lower than the proportion of tumor-forming cells identified in NSG mice (Fig. 1a). Notably,