Bony Endothelium: Tumor-Mediated Transdifferentiation?

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In this issue of *Cancer Cell*, Dudley et al. (2008) report that endothelial cells (ECs) from murine prostate tumors unexpectedly differentiate into cells with characteristics of bone and cartilage and that human and murine prostate tumors contain "calcified" ECs. These observations open the possibility for targeted antiangiogenic therapy.

Endothelium is a mesodermal derivative that codevelops with hematopoietic stem cells (HSCs) from so-called hemangioblasts (Habs) located in the dorsal aorta (Medvinsky and Dzierzak, 1996) (Figure 1). A second precursor for endothelium also isolated from the dorsal aorta, termed mesoangioblasts (Mabs), generates endothelial cells (ECs) as well as extravascular mesodermal derivatives including skeletal muscle, bone, and cartilage, but not HSCs (Peault et al., 2007) (Figure 1). In postnatal life, Mabs with differentiation potential similar to fetal Mabs have been isolated from numerous tissues even though postnatal Mabs generally express pericyte rather than EC markers found on fetal Mabs (Peault et al., 2007). No good evidence exists that Habs persist postnatally.

In the developing embryo, blood vessels are formed via an initial process of vasculogenesis followed by remodeling of the vessels in a process termed angiogenesis, giving rise to a complex blood vessel system consisting of arteries, capillaries, and veins. ECs are specified depending on the vessel type (vein, artery, or capillary) to which they belong (Chi et al., 2003) (Figure 1). Capillaries of the microvasculature consist essentially of ECs surrounded by support cells, the pericytes. ECs found in different capillary beds are also specified: differences in structural and functional properties as well as cell surface antigens and transcriptome have been described for capillary ECs from different organs (Langenkamp and Molema, 2008). ECs thus appear to be highly adapted to the surrounding tissue. Owing to their specification, capillary ECs play unique roles in pathological conditions specific to the organ in which they reside.

New blood vessel formation in postnatal life occurs chiefly via angiogenesis. Only recently has it become apparent that vasculogenesis, or the recruitment of EC-restricted progenitors called endothelial progenitor cells (EPCs) to newly formed vessels, also plays a role in neovascularization (Lyden et al., 2001). Whether by activation of local postcapillary ECs or maturing EPCs, the initial event underlying vascularization of (for instance) tumors is the invasion of ECs in the tumor mass to form primitive vascular tubes, which then attract pericytes to generate the surrounding supportive layer. As is true for ECs present in normal tissues and organs, the phenotype of tumor ECs (TECs) varies significantly in response to the surrounding tumor microenvironment (Langenkamp and Molema, 2008).

In this issue of *Cancer Cell*, Dudley and colleagues (2008) report that ECs isolated by clonal culture from spontaneously developing prostate tumors in mice generate blood vessels in vitro and in vivo in Matrigel plugs, as expected. Unexpectedly, a significant increase in genes and proteins characteristic of bone and cartilage was seen following exposure of the clonally isolated TECs to osteogenic or chondrogenic culture conditions, respectively, which was not seen in ECs isolated from dermis. TECs expressed a number of typical EC antigens, such as CD31, VEGFR-2, and vWF, but not others, such as CD34 and SCA-1. Interestingly, TECs expressed antigens not found in mature ECs, such as CD133 and CD90. CD90 was also expressed in mesenchymal stem cells (MSCs), but mouse MSCs also expressed SCA-1. Primary human and murine prostate tumors often contained areas of calcification. The authors found that approximately 4% of $CD31^+$ human prostate tumor blood vessels were positive for von Kossa staining, which detects areas of high concentrations of inorganic phosphate typical of calcification. Although calcification has been observed in vessel walls, it usually is located in the smooth muscle layer surrounding arteries and not at the luminal side of vessels.

As discussed above, ECs are highly adaptable, and their phenotype can be influenced by the tumor environment. The osteogenic tumor microenvironment typical for prostate cancer could therefore be responsible for the calcification observed in prostate TECs. However, ECs are lineage restricted and do not normally generate other mesodermal cell types. So, what is the mechanism underlying this novel observation?

One possibility is that cells recruited to the vascular bed of (prostate) tumors are not EC-restricted EPCs but cells with a broader differentiation ability, such as Mabs, among others (Figure 1). Aside from Mabs, a number of other, perhaps related, cell populations have been isolated from muscle that have the ability to generate not only skeletal myoblasts but other extravascular mesodermal cells and endothelium, including culture-isolated muscle-derived stem cells (MDSCs) (Peault et al., 2007) and prospectively isolated myoendothelial cells (Peault et al., 2007). Moreover, a number of cell populations have been isolated from cultured bone marrow, umbilical cord blood, or

other tissues that differentiate into endothelial cells, aside from cells with not only mesodermal but also endodermal or ectodermal features (Serafini and Verfaillie, 2006). The role of these cells in neovascularization processes is unknown even though many of these non-EC-restricted stem/progenitor cells contribute to some degree to vessel formation in ischemic lesions or tumors when administered exogenously. As Dudley et al. (2008) have done, one could determine whether the TECs have antigenic determinants in common with any of these cell populations. However, the ability of ECs to adapt to their environment is exemplified by the ease with which they adapt themselves to culture conditions. Because of this context-dependent adaptation, in vitro-cultivated ECs may not fully reflect the antigenic determinants found in vivo (Langenkamp and Molema, 2008).

Another possibility, also suggested by the authors, is that under certain circumstances such as the presence of tumor cells, ECs can acquire unexpected differentiation potential. Recent studies have suggested that for a number of epithelial tumors, genetic mutations lead to the reacquisition of features of stem cells by progenitor or precursor cells, a process referred to as dedifferentiation (Kasper, 2008). Moreover, in the era of induced pluripotent stem cells, it has become obvious that a differentiated state can, at least in vitro, be changed to a more multi/pluripotent state (Takahashi et al., 2007). One could hence speculate that the ability of TECs to differentiate at least to some extent into cells with osteogenic and chondrogenic properties has been evoked by the tumor microenvironment, leading to the reacquisition of a more broad mesodermal fate (Figure 1).

Direct transdifferentiation, without the acquisition of a more primitive state, has been described for a number of types of cells, including MSCs and cells in the pancreas, among others (Caplan, 1991; Lardon et al., 2004). It is therefore also possible that some TECs undergo direct transdifferentiation from an EC to an MSC-like cell (Figure 1). Another possibility is that clonally derived, cultured TECs have been dedifferentiated by the culture process and therefore have acquired broader potential, as might be the case for some of the non-EC-restricted cells capable of generating endothelium as discussed above (Serafini and Verfaillie, 2006). The in vitro studies compared TECs from prostate with capillary ECs from dermis; thus, the difference in the microvascular bed from which the cells were selected could also play a role in the differences in phenotype and/or differentiation potential of the ECs. However, as $CD31^+$ cells in capillaries in primary prostate tumor samples contain inorganic phosphate, as shown by the von Kossa staining, the ability of $CD31^+$ cells to produce calcified deposits is not merely a characteristic acquired in vitro.

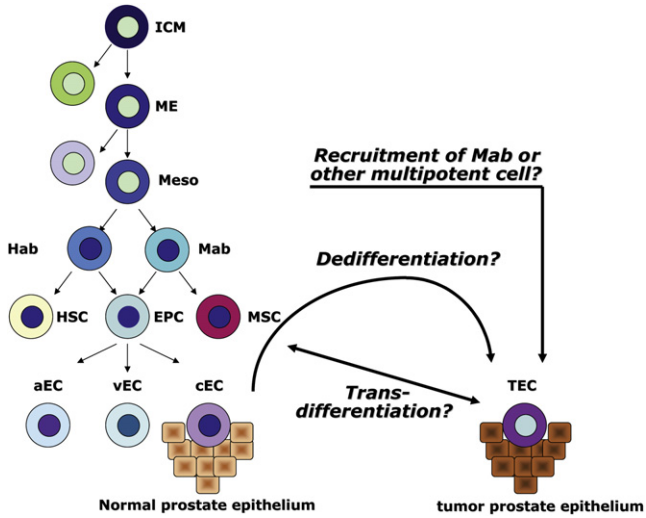

Figure 1. Possible Mechanisms Underlying the Apparent Osteogenic Differentiation of Tumor Endothelial Cells

Endothelial cells (ECs) are derived from mesoderm (Meso) and may appear from either a common endothelial/hematopoietic progenitor, termed hemangioblasts (Habs), or another endothelial/extravascular mesodermal progenitor, termed mesoangioblasts (Mabs), both present in the floor of the aorta. Via asymmetric divisions, the two common progenitors would give rise to hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs), respectively, plus endothelial progenitor cells (EPCs). EPCs differentiate into ECs that are specified depending on the blood vessels to which they populate: arterial ECs (aECs), venous ECs (vECs), or capillary ECs (cECs). cECs are further specified depending on the microvascular network they belong to. Dudley et al. (2008) have identified tumor endothelial cells (TECs) that can differentiate into cells with osteogenic and chondrogenic characteristics. This may reflect the recruitment of endothelial/extravascular mesodermal progenitors to the tumor vascular bed, tumor-mediated dedifferentiation of cECs into cells that have endothelial/extravascular mesodermal characteristics, or transdifferentiation between ECs and MSC-like cells. ICM, inner cell mass; ME, mesendoderm.

It will be of interest to study tumor vasculature in other types of tumors to determine whether TECs can adapt themselves to the tumor environment by acquiring some of the characteristics of that specific environment. Such specification may ultimately allow one to specifically target the TECs, but not normal ECs, to treat tumors. The question of whether this represents a spontaneous in vivo dedifferentiation or the recruitment of non-EC-restricted stem/progenitor cells as a source of TECs can only be resolved by clever lineage-tracing experiments.

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