Previews

Reenthronement of the Muscle Satellite Cell

In this issue of *Cell* (Sherwood et al., 2004), a quantitative survey confirms that repair of skeletal muscle is overwhelmingly attributable to the endogenous satellite cell population but that experience of a regenerating muscle environment confers some myogenic qualities onto a tiny population of bone marrow-derived cells.

Viewed historically, skeletal muscle would appear an unlikely arena for the debate on stem cell plasticity. From its discovery in 1961, the satellite cell, nestling between the fiber surface and the overlying basement membrane, has been the almost uncontested candidate as the muscle precursor or tissue specific stem cell. It has remained so until recent challenge of its status by a string of demonstrations that cells derived from genetically marked bone marrow grafts can contribute to regenerating skeletal muscle (Ferrari et al., 1998; Gussoni et al., 1999; LaBarge and Blau, 2002). That this has developed into a lively debate, punctuated by minor skirmishes, has more to do with differences in interpretation and emphasis than with truly discrepant results between the proponents of the various schools of thought. Now, a paper by Sherwood et al. (2004) cuts a swathe through the main areas of altercation, effectively reinstating the satellite cell as the main player in the mechanism of regeneration of skeletal muscle and reducing the issue from grand arguments over fundamental biological principles to quibbles over minor issues. It does however redeem some of the grounds for argument and further investigation by identifying some fascinating features of the routes by which the occasional bone marrow-derived cell finds its way into the regenerating muscle fiber. In addition, it raises new questions about recent proposals of a resident muscle stem cell situated outside the classical satellite cell compartment.

Telling findings are based mainly on a two-phase enzymic digestion of whole muscle, resulting in segregation into the readily dissociated "interstitial" cells and the "fiber-associated" cells released during the second phase. This protocol has the virtue of providing cells in sufficient numbers for a range of analytical and preparative FACS sorts on the basis of antigenic and lineage markers. This permits a degree of quantitative comparison between cell populations with the result that some perspective can be put on the relative importance of the functional groups described. Its main limitation is that the two main populations cannot be linked precisely to any histologically defined cell compartment. In practice, this presents no major impediment to broad interpretation and a number of clear points emerge from this study, notably in the analysis of the activities of cells from GFP-marked bone marrow in non-GFP transgenic hosts versus those that do not carry the marker of bone marrow origin.

The upshot is that a single population is identified as substantially myogenic according to the criteria applied: namely spontaneous differentiation in tissue culture, expression of muscle-specific genes on coculture with myogenic cells, and participation in myogenesis on transplantation into regenerating muscle. This population is a subset of the fiber-associated cell that does not carry the GFP bone marrow marker and is thus of endogenous muscle origin. These cells belong to the sorted subset that are $CD34^+Sca1^-$, a phenotype that corresponds well to the preactivated satellite cell on isolated muscle fibers (Zammit and Beauchamp, 2001). The other major fiber associated population, $CD34^+Sca1^+$, forms fibroblast-like colonies in culture and does not contribute to muscle regeneration in vivo, corresponding phenotypically to the microvessel associated cells (Zammit and Beauchamp, 2001)

In line with previous studies using transgene-marked bone marrow, cells of bone marrow origin are found in both fiber-associated and interstitial categories extracted from either regenerating or nonregenerating muscle. However, these two environments exert their own distinct effects on the behavior of the bone marrow-derived cells that take up residence within them. In both cases, populations are found bearing a range of antigenic profiles, including some corresponding to those found on resident GFP^- cells, but the functional characteristics of these cells differ according to whether or not the muscle had been injured. GFP^+ cells from noninjured muscles showed little or no myogenic function by any of the tests, whereas cells from the same sorting windows, derived from muscles injured two days previously, were found to show minor but distinct propensities for some degree of myogenic differentiation by one or more of the test criteria. Whether this change is generated by an educational process or by selection of cells with such competence from the general bone marrow-derived population within regenerating muscle is clearly of interest. Intriguingly, such cells are generated within animals grafted with whole bone marrow but not with hematopoietic stem cells. This functional change is not restricted to simple ability to express muscle genes and to become involved in muscle regeneration if injected into a damaged muscle, for the fiber-associated cells showed a predilection to reenter this compartment on reinjection into such a muscle.

At the time of its discovery, the fact that circulating bone marrow-derived cells could contribute to various tissues, including skeletal muscle, generated considerable excitement because both of its therapeutic promise and its fundamental biological implications. It held hope of fulfilling the would-be cell therapist's dream of dispersed distribution of multipotent cells from a bone-marrow reservoir to sites of muscle repair (Partridge, 2003). It was also suggested that delivery via the blood vascular route might be a significant arm of the mechanism of normal maintenance of tissue-specific precursors, constituting the "stem cell highway" (Blau et al.,

2001). This ran counter to earlier findings based on a less sensitive marker (Grounds, 1983) and is flatly contradicted by the Sherwood study, but the sporadic entry of cells derived from whole bone marrow grafts into the myogenic process, seemingly by a number of pathways, is vindicated, and sustains some hope of therapeutic applicability. This study also failed to confirm the thesis that CD45^{+} cells residing in muscle act as a significant source of myogenic stem cells, (Polesskaya et al., 2003) but the original claim was made on the basis of a four day postinjury model rather than the two day setup used here, and one should be wary of the transience of expression of some of the markers used in the majority of these studies. Doubt as to the applicability of general principles between species must also be born in mind, especially in view of the recent description of what appears to be a robust myogenic activity in vivo among a tiny minority population of circulating cells in human blood (Torrente et al., 2004).

Undoubtedly, the main impact of the Sherwood paper is to consolidate the view that the contribution of cells derived from the bone marrow into regenerating skeletal muscle under normal physiological circumstances is trivial. Although it does not completely invalidate the notion of therapeutic application of this mechanism, it certainly provides no encouragement. However, the fact that some bone marrow cells can be persuaded into the myogenic pathway remains as a tantalizing conundrum. What is the nature of this phenomenon? In itself, it is too ineffectual to be selectable by Darwinian mechanisms. Could it be an ancillary of some more biologically important process or is it simple biological "noise" within the mechanisms of differentiation?

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Selected Reading

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HDAC4: A Corepressor Controlling Bone Development

RUNX2 is a transcription factor with a well-characterized role in bone development. In this issue of *Cell*, Vega and colleagues (Vega et al., 2004) show that HDAC4 interacts with RUNX2 and impacts upon chondrocyte hypertrophy and bone formation.

Histone deacetylases (HDACs) are transcriptional coregulators with the capability of modifying chromatin structure and other transcription factors. HDACs fall into two general classes based on sequence homology and domain structure. Class I HDACs have homology to yeast RPD3 and lack an N-terminal domain present in class II proteins. Class II HDACs are homologous to yeast HDA1 and have an N-terminal extension that regulates activity. HDACs are emerging in vital cellular processes including growth, differentiation, and apoptosis, among others. They are of interest as pharmacological targets in cancer and in cardiac pathology. Because of their widespread importance and enormous clinical potential, a clear understanding of their functions in vivo is urgent.

The Olson laboratory has made dedicated efforts to characterizing the class II HDACs. Recently, they demonstrated a critical role in modulating the growth response of cardiac muscle to work (Chang et al., 2004; Zhang et al., 2002). Loss-of-function in HDAC5 or HDAC9 results in cardiac hypertrophy in unchallenged mice, and the deficiencies cause hypersensitivity to cardiac stress such as banding of the thoracic aorta or ectopic expression of calcineurin. Conversely, a gain-of-function mutation in a product of the HDAC9 gene inhibits cardiomyocyte hypertrophy in vitro. The phenotypic effects of the class II HDACs are, at least in part, associated with MEF2, a transcription factor connected with cardiac hypertrophy. Thus, two class II HDACs have in vivo roles in a pathway controlling cardiac cell growth, and their activity is mediated by a key DNA binding transcription factor.

In this issue of *Cell*, the Olson laboratory proposes that HDAC4 plays a role in chondrocytes analogous to HDACs 5 and 9 in cardiomyocytes (Vega et al., 2004). They support their arguments using a combination of loss-of-function and gain-of-function models. The loss-of-HDAC4 model was engineered by deleting the sequence encoding the MEF2 binding domain. The mutation accelerated endochondral bone formation. Mice displayed premature ossification of multiple cartilaginous sites and even formed bone in regions like the costochondral cartilage that normally do not become ossified. By contrast, HDAC4 gain-of-function slowed the ossification of cartilage in vivo. Intramembranous bone formation was normal, consistent with HDAC4 involving cartilage development. The process of bone formation from cartilage involves a stage of chondrocyte hypertrophy, and in situ analysis of HDAC4 showed that expression was upregulated in prehypertrophic and hypertrophic chondrocytes of ossifying ribs. It is this hypertrophic phase of development that the authors believe to be critically influenced by HDAC4.