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RESEARCH HIGHLIGHT

Hematopoietic potential cells in skeletal muscle

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During mouse embryogenesis, the formation of primitive hematopoiesis begins in the yolk sac on embryonic day 7.5 (E7.5). Thereafter, definitive hematopoietic stem cell (HSC) activity is first detectable in the aorta-gonad-mesonephros (AGM) region on E10, followed by fetal liver and yolk sac. Subsequently, the fetal liver by E12 becomes the main tissue for definitive hematopoiesis. At a later time, HSC population in the fetal liver migrates to the bone marrow, which becomes the major site of hematopoiesis throughout normal adult life [1]. It remained unclear whether hematopoietic stem/progenitor cells exist in non-hematopoietic tissues. In 1980s, Bartlett [2] first described that adult mouse brain contained significant amount of colony forming units (CFU-s). The average number of CFU-s obtained per 10^5 dissociated adult brain cells was significantly higher than other adult tissues such as lung, kidney, heart, thymus and blood. However, Hoogerbrugge *et al*. [3] could not obtain such high number of CFU-s in adult brain and concluded that the CFU-s observed by Bartlett in preparations of mouse brain did not originate from the brain tissue. Nevertheless, recent work revealed that hematopoietic stem/progenitor cells clearly exist in several tissues besides bone marrow [4, 5]. For example, adult liver has been shown to contain HSCs that reconstitute whole hematopoiesis in lethally irradiated animals. HSCs and hematopoietic progenitor cells readily colonize the adult spleen. In addition, adult lung contains large numbers of alveolar macrophages derived from progenitors identified in fetal lung. Furthermore, T cell differentiation occurs in extra-thymic sites, such as intestine and liver.

In 1999, Gussoni *et al*. demonstrated that adult skeletal muscle contains side population (SP) cells which have the potential to give rise to hematopoietic cells in lethally irradiated mice following intravenous injection [6], indicating that muscle SP fraction contains muscle hematopoietic potential cells (HPCs). In 1996, Goodell *et al*. first discovered that HSCs in bone marrow from many different species can be isolated in SP cells by fluorescence activated cell sorting (FACS) [7]. The SP cells exclude Hoechst 33342 DNA-binding dye through the activity at the cell surface by multi-drug resistance (MDR) pump proteins such as ABCG2/BCRP1 (reviewed in reference [5]). Jackson *et al*. and other groups reported that cultured dissociated muscle-derived cells display the capacity to reconstitute the entire hematopoietic repertoire following intravenous injection into lethally irradiated mice, suggesting that muscle HPCs can be expanded *ex vivo* while maintaining their hematopoietic stem cell activity (reviewed in reference [5]). In addition, *in vitro* hematopoietic colony formation assays demonstrate that adult muscle contains a remarkably high level of hematopoietic progenitors that differentiate into multiple types of hematopoietic colonies [4, 8-11], reviewed in reference [5] and Figure 1. These muscle HPCs can also be enriched in the muscle SP fraction, as they are in bone marrow-derived SP cells [4, 11]. These observations raised an interesting question about the origin of these

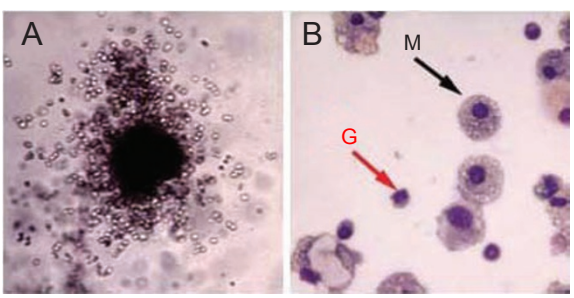

Figure 1 Adult muscle contains hematopoietic progenitors. **(A)** Large tight colony was formed in culture of muscle-derived cells. **(B)** May-Grunwald's Giemsa staining. Arrows indicate a monocyte (M) containing some vacuoles and a granulocyte (G) with a polymorphic nucleus, respectively.

muscle HPCs. The answer seemed relatively simple since only $CD45^+$ muscle-derived cells display the capacity to give rise to hematopoietic cells *in vitro* and reconstitute the entire hematopoietic repertoire following intravenous injection into lethally irradiated mice [8, 10, 12] and reviewed in reference [5]. In addition, bone marrow transplantation experiments demonstrate that muscle HPCs are indeed of bone-marrow origin. Therefore, circulating HPCs originating from bone marrow may reside within skeletal muscle during development. For instance, marrow-derived cells migrate into skeletal muscle via hepatocyte growth factor (HGF) and c-met interaction [13]. However, recent experiments demonstrate that primate skeletal muscle appears to lack the ability to retain long term repopulating HSCs [14]. The reason for this lack of functional conservation between species is unclear.

Not only muscle but also many other adult tissues, such as brain, heart, lung, liver, spleen, kidney, and small intestine, contain different amount of HPCs that can also be enriched in the $CD45^+$ and SP fraction [4]. These adult tissue-derived HPCs are not simply contaminating peripheral blood cells within the tissue preparation, rather than residing within the tissues [4, 8]. Therefore, these HPCs are normal residents in many adult tissues. However, hematopoietic reconstitution of irradiated mice has been tested only for cells isolated from adult skeletal muscle and liver, which exhibit long term HSC activity reviewed in reference [5]. Therefore, whether other adult tissue-derived HPCs exhibit the potential of hematopoietic reconstitution of irradiated mice remains an interesting question. *In vitro* assays demonstrate that bone marrow, skeletal muscle, spleen and liver appear to contain more undifferentiated multipotential myeloid progenitors, than the other tissues, including brain, heart, lung, kidney, and small intestine, which seem to contain more committed myeloid progenitors, such as macrophages and granulocytes [4] and reviewed in reference [5]. This observation implies that there is something unique about the skeletal muscle niche that allows it to support the survival and maintenance of the HPCs. However, it remained unclear whether there are any functional differences between muscle- and marrow-derived HPCs and whether clonal muscle-derived HPCs from the $SP/CD45^+$ population have the multilineage differentiation ability.

The paper by Haond *et al*. in a recent issue of Cell Research [15] quantified hematopoietic progenitor/stem cell activities of murine bone marrow-, muscle- and blood-derived $CD45^+$ or $SP/CD45^+$ cells *in vitro*. Haond *et al*. found that there are significant phenotypic differences in muscle $CD45^+$, marrow $CD45^+$ and blood $CD45^+$ cells as analyzed by several cell surface markers, including hematopoietic stem cell markers, such as Sca-1, c-kit, Thy-1, and markers for lymphocytes and myeloid cells. Clonogenic efficiency of muscle $CD45^+$ cells was enriched into $SP/CD45^+$ fraction. Interestingly, muscle $SP/CD45^+$ cells contain multipotential long-term culture-initiating cells (LTC-IC) and the frequency is much higher than that of blood-derived cells but relatively lower compared to bone marrow-derived cells. Single-cell-sorted muscle $SP/CD45^+$ cells displayed robust proliferative activity, especially after culture with both cytokines and OP-9 stromal cell layers which can also support hematopoietic stem cell differentiation from embryonic stem (ES) cells. These amplified clonal cell population displayed the multilineage differentiation capability, including myeloid, lymphoid and NK cells. This is the first demonstration that, similar to bone marrow-derived cells, a single cell in muscle SP fraction exhibits major proliferative potential and multilineage differentiation capability.

The reason why muscle contains HPCs that possess such a remarkable capability for hematopoietic differentiation potential remains an interesting question. Nevertheless, the presence of HSCs-like stem cells in adult muscle raises the possibility that such stem cells locally contribute to host tissues when exposed to the correct environment during regeneration. Clearly, further experimentation is required to investigate the origin and biological significance of the HPCs within non-hematopoietic tissues.

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