Development of blood cells in the ascidian *Halocynthia roretzi*

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In recent years our understanding of the ontogenesis of mammalian hematopoietic cells has been improved greatly. However, we have only a limited knowledge about the ontogenesis of ascidian hemocytes. The trunk lateral cells (TLCs) of the tail bud embryo are the origin of hemocytes and body wall muscle in the ascidian *Halocynthia roretzi*. The TLCs migrate posteriorly and ventrally, and differentiate to hemocytes and muscle cells in the juveniles. Each ascidian species has nearly ten types of hemocytes in adulthood. It has been shown repeatedly that the hemocyte types of adults are very variable among ascidian species, and this fact made difficult to reveal the relationships of the hemocyte types between species. All ascidians ever studied, however, have a common type of hemocytes, the hemoblast. They are undifferentiated cells having typical morphology with a conspicuous nucleolated nucleus and scanty cytoplasm. The hemoblasts are thought to be hematopoietic stem cells (HSCs). Although the hemoblasts are found in the hematopoietic tissue and in circulation, it is not clear that both of them are truly HSCs.

In this study, the development of hematopoiesis was studied in detail with electron microscopy in the ascidian *H. roretzi*. We found only four types of hemocytes in the juveniles, and the scarcity of the hemocyte types allowed us to follow the process of their differentiation distinctly. In the swimming larvae and juveniles, nascent mesoderm cells infiltrate between the epidermis and endoderm. The mesoderm cells have yolk granules and compact masses of glycogen granules, and those nutritive granules are gradually consumed after metamorphosis. Consequently, in 5d juveniles, the mesoderm cells transform to hemoblasts with a scanty cytoplasm and a conspicuous nucleolated nucleus. Before the appearance of the hemoblasts, hematopoiesis initiates soon after metamorphosis. This fact means that the mesoderm cells directly differentiate to giant cells, vacuolated cells, and granular cells. About 5 days after metamorphosis the hemoblasts begin to differentiate to the three types of hemocytes instead of the mesoderm cells. The proportion of hemocyte types in the juveniles is very different from that in adults, for example the hemoblasts and giant cells are rare in the adults but they are major hemocyte types in the juveniles. The hemoblasts seem to be rapidly dividing cells because we found their mitotic figures in the hemocoel of the juveniles. We concluded that the circulating hemoblasts are HSCs that self-renew and replenish multiple types of hemocytes in the ascidian *H. roretzi*.

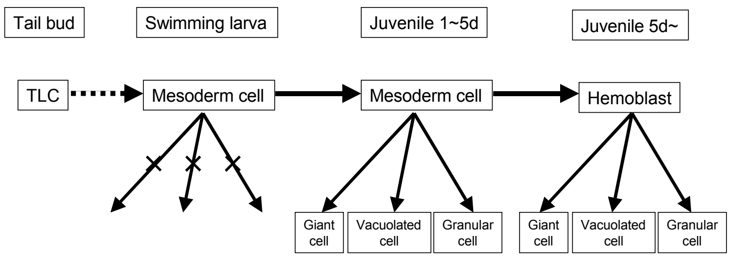

Figure shows the ontogenesis of hemocytes revealed in this study in the ascidian *H. roretzi*. Nascent mesoderm cells never differentiate to hemocytes in the swimming larvae. They begin to differentiate to three types of hemocytes in the juveniles after metamorphosis. Prior to the 5th day after metamorphosis, the nascent mesoderm cells transform to hemoblasts. Thereafter the hemoblasts differentiate to the three types of hemocytes, that is, giant cells, vacuolated cells, and granular cells. All these results distinctly show that the nascent mesoderm cells in the juveniles are multipotential cells. In addition, it is evident that the hemoblasts, derived from the nascent mesoderm cells, are HSCs.