OVARIAN STRUCTURES THAT SUPPORT REPRODUCTIVE CYCLES- GERMLINE STEM CELLS AND THEIR NICHE STRUCTURE IN OVARY

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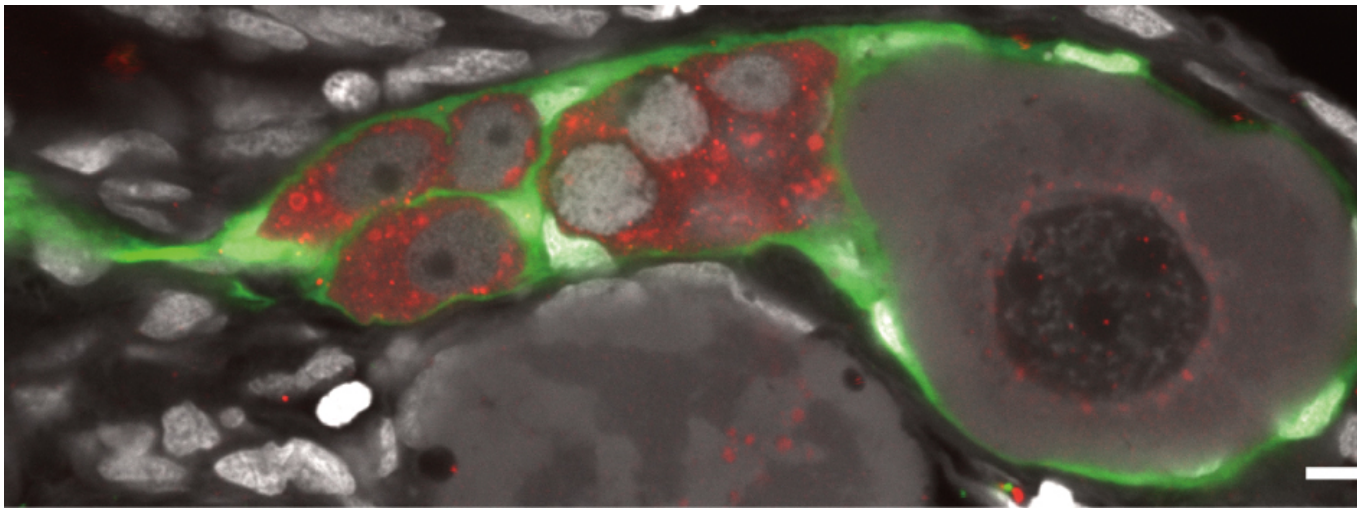
Fish species form a largest group in vertebrates and different species show a variety of reproductive cycles, dependent on their reproductive strategies. Some species spawn eggs everyday but another species exhibit periodical spawning over years. From the intensive analyses of histology, it has been thought that the regulation at the level of oogonia and spermatogonia is a key to proper reproductive cycles. However, little is known about the characters of these gonial cells in the process of production of eggs and sperms.

Stem cells are the conceptual cells that have the capability both to keep producing differentiated cells and to retain self-renewal. In the reproductive organs, there should be many types of stem cells that could explain not only the continuous production of eggs and sperms (germline stem cells) but also the maintenance and growth of its structure (somatic stem cells). Especially in mammals, germline stem cells have been investigated intensively. Recent research has proved the presence of germline stem cells in testis and begins to reveal their characters and regulations while it is well accepted that there are no germline stem cells in ovary because all primordial germ cells in the developing ovary differentiate into oocytes and make a pool of oocytes during fetal stages for adult reproductive term. Therefore the presence of germline stem cells that accounts for many aspects of reproduction in ovary has not been identified in vertebrates.

Medaka (*Oryzias latipes*) spawns a dozen of eggs everyday at the onset of light during a couple of months of reproductive term. Our previous analysis indicated that *nos2* gene is expressed in both oogonial and spermatogonial cells and that, unlike mammalian gonads, medaka *sox9* orhtologue, *sox9b*, is expressed in both ovary and testis. With combination of heat-inducble cre recombinase and *nos2*-promoter driven fluorescent protein (GFP and RsRed), we have developed transgenic medaka that allow clonal analysis of germ cells. This analysis clearly indicated that *nos2*-expressing oogonia have the ability to produce eggs continuously for a reproductive term and these eggs are found fertile to develop into embyos. Therefore the *nos2*-expressing oogonia completely fulfill the criteria for germline stem cells.

The germline stem cells reside in a special structure, called germinal cradle, composed of *sox9b*-expressing cells (Figure 1). The germinal cradles are interconnected to each other with a thin cellular process of *sox9b*-expressing cells, thus forming networks, called ovarian cords. This networks spread within germinal epithelium on the dorsal surface of ovary. The germinal epithelium is the epithelial tissues where many researchers more than 50 years ago postulated the presence of oogonia in adult mammalian ovary and one of the important tissues

Figure 1. An ovarian niche for germline stem cells (germinal cradle): Red cells are germ cells at the early stages of oogenesis and green cells are *sox9b*-expressing supporting cells



for the origin of ovarian cancer. Oogenesis proceeds from germline stem cells to very early stages of oocytes in the germinal cradles. Cystic division of amplifying oogonia occurs in the cradles, which is evidenced by imaging technique. The imaging also reveals that some populations of oogonia are eliminated during the cystic division. All these observations indicated that a germinal cradle is a very important place to ensure and regulate reproduction according to both internal and external environment. The sex of medaka is determined genetically. However, some treatment, for example causing the change of hormonal environment, exerts sex reversal. In addition, it is indicated by other group that germ cells are sexually reversible or bipotent. These evidences suggest that the germinal cradles are also important for regulation and maintenance of sex.

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