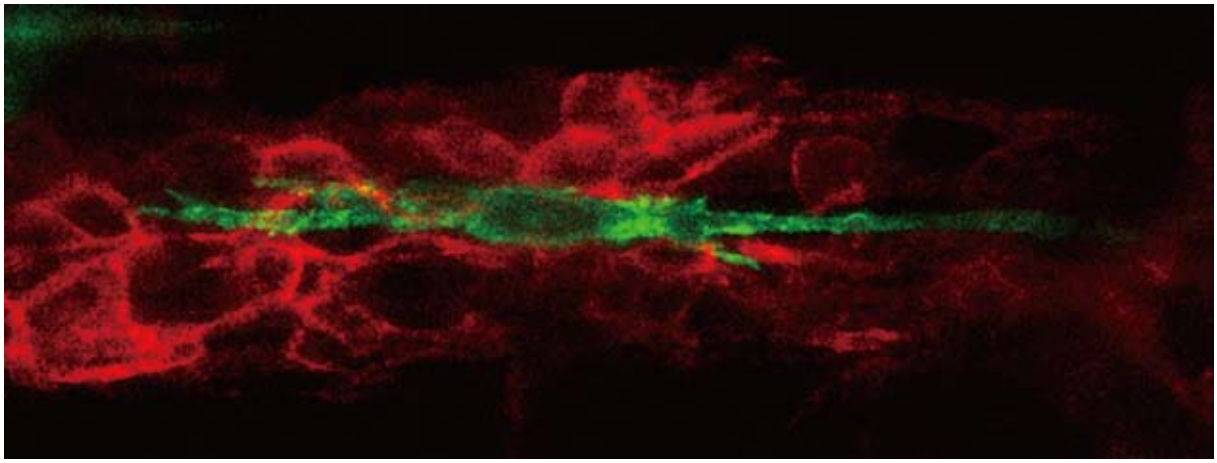
Identification of Multipotent Mammary Stem Cells Fueling Hope for New Targeted Therapy of Breast Cancer

By SONG Jianlan (Staff Reporter)

A team of researchers at the Shanghai Institutes for Biological Sciences (SIBS), CAS reported Oct 19 online in *Nature* the successful identification of a long sought-after type of stem cells, the multipotent mammary stem cells (MaSCs) in mouse mammary gland, through a surface marker specifically expressed by this type of stem cells. This discovery settles a raging debate on the existence of multipotent MaSCs that can differentiate into all types of mammary epithelial cells, and moreover provides an ideal target for new drugs against breast cancers, especially a subtype that responds to no existing targeted therapy.

Multipotent vs Unipotent

Apart from embryonic stem cells, another type of stem cells called adult/tissue stem cells can be found in various tissues of mammals. These stem cells are vital for the development, regeneration and maintenance of relevant tissues. In the mammary gland there exist two types of epithelial cells, namely basal (myoepithelial) and luminal cells. Previous research indicated that there might exist a kind of multipotent MaSCs on the basal layer of the epithelium, however no effort succeeded in singling out this versatile type of stem cells from the heterogeneous population. Instead, in 2011 two types of unipotent MaSCs were found responsible for the regeneration and maintenance of the basal and luminal cells respectively. Hence a theory rose, arguing that multipotent MaSCs were found only during embryonic development of the mammary gland, while in postnatal development of the gland only two



A SIBS team successfully identified the long sought-after multipotent mammary stem cells (MaSCs, showed with green fluorescence) from the differentiated cells (with red fluorescence) on the basal layer of the epithelium. (Image by courtesy of Dr. ZENG)

types of lineage-restricted unipotent MaSCs present. As a result, whether or not multipotent MaSCs exist on the top of the cellular hierarchy in the adult gland became highly controversial.

Now the team led by Dr. ZENG Yi at the Institute of Biochemistry and Cell Biology (IBCB), SIBS has brought this debate to an end. In cooperation with Dr. YANG Li at the CAS-MPG Partner Institute for Computational Biology (PICB), SIBS, Dr. ZENG's lab first identified in their microarray analysis of cultured MaSCs a surface protein, namely the Protein C receptor (Procr), as a specific product of a candidate of multipotent MaSCs. Further, with help from this marker receptor they successfully isolated the Procr-expressing cells and verified their regenerative ability and multipotency through strict transplantation assays and linage-tracing studies, demonstrating that this Procr-expressing cells IS a type of multipotent MaSCs that have very good ability of repopulation and can differentiate into all kinds of mammary cells.

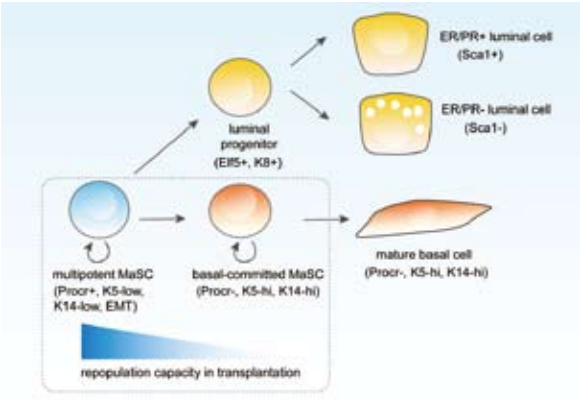
Novel *In Vitro* System

What has played an instrumental role in the team's investigation is a novel *in vitro* system capable of maintaining the MaSCs' properties, based on the previous work by ZENG and Prof. Roel Nusse, supervisor of her postdoctoral research at Stanford University. They discovered in 2010 that the Wnt proteins, a diverse family of signaling glycoproteins, can expand MaSCs and maintain their stem cell status in three-dimensional Matrigel culture, indicating that certain Wnt members are crucial for determining the stem cell properties. In an effort to optimize the *in vitro* system, ZENG identified that Wnt4 together with its potent agonist R-spondin1 (Rspo1) form the Wnt signaling pathway for the maintenance of MaSCs. This work by her group was published on Sept 26 this year in *Genes & Development*, as detailed later in this report.

Based on these discoveries, the *in vitro* system has made it possible for researchers to observe, trace and screen different compositions on the basal layer of the epithelium, including the multipotent stem cells of interest, progenitors produced by the stem cells, and mature basal epithelial cells, all independent of the physiological situation of mice.

With aid from this *in vitro* system, they searched for candidate marker genes of multipotent MaSCs among the Wnt signaling targets whose expressions are increased in presence of Wnt proteins, considering the important role of Wnt signaling in MaSC's self-renewing. What they finally chose was the Procr. Following the Procr-expressing genes they successfully identified and isolated the stem cells.

Subsequent transplantation assays demonstrated



The team successfully demonstrated that the Procr$^+$ MaSC (blue) is at the top of the cellular hierarchy, followed by differentiate cells (yellow). (Image by courtesy of Dr. ZENG)

that Procr-expressing cells have the highest repopulation efficiency compared with control groups; and linage-tracing studies showed that they are multipotent. Therefore the team concluded that among different epithelial cells in the mammary gland, this type of cells locates at the top of the cellular hierarchy and coexists with the two linages of unipotent MaSCs found before.

Possible Link to "Triple-Negative" Breast Cancer

The finding might also boost a new round of re-evaluation of the initiation of breast cancer subtypes, and hence have implications in further research on breast cancers as well as their treatments.

Breast cancers could fall into four different subtypes, depending on which of the three important types of receptors they have: estrogen receptors (ER), progesterone receptors (PR), or HER2. For example, if the subtype has ER, it is called ER$^+$ breast cancer. Drugs are available targeting these three receptors, therefore subtypes expressing the three receptors can be treated with targeted therapies, and hence have improved prognosis.

However, for the fourth subtype that does not express any of the three receptors, namely the triple-negative breast cancer, no targeted therapy is available. According to the researchers, about 20% breast cancers fall in this subtype and have the highest metastasis rate. Patients suffering from this subtype can only be treated with conventional chemotherapy and radiotherapy, and have the worst three-year survival rate.

The difference in receptor status across subtypes, however, suggests different origins of the subtypes. They could have evolved from abnormalities in different types of

stem cells. The multipotent MaSCs identified by the team do not express any of the above-mentioned receptors either. This similarity might suggest its possible role in initiation of the triple-negative subtype of breast cancer, and might shed some new light on its treatments.

"Lesion in the multipotent MaSCs might have some correlation with the triple-negative breast cancer," ZENG comments: "We are still working on this to make it clear."

New Hope for Targeted Therapies

Thankfully the triple-negative subtype expresses Procr, and this gives us a handle on the concept of new drugs against this subtype. "The discovery of this marker receptor offers new hope for treatments of triple-negative breast cancer. Moreover, Procr is a transmembrane protein – it seats on the cell surface," introduces ZENG: "This means it can be an ideal target for future drugs, as properly designed chemicals can bind to the receptor and cause desired changes in the cancerous cell without penetrating through the cell membrane."

For this sake, ZENG's team has launched cooperation with some pharmaceutical companies. When asked how long it will take to develop such new drugs, ZENG answered with caution: "I can't make any prediction now. This is just a result of fundamental research, still a long way from clinical applications."

ZENG has been focusing her research on the regulatory signals of MaSC self-renewal and the interaction between stem cells and their niche, particularly how the self-renewal is maintained. In the work mentioned earlier published in *Genes & Development*, her team revealed a hormone-induced collaborative local niche environment of MaSCs that contributes to the latter's expansion. They successfully identified the Rspo1 as a novel hormonal mediator in the mammary gland. Working with another hormonal mediator, Wnt4, the Rspo1 promotes self-renewal of the MaSCs through a signaling pathway featuring Wnt family of signaling glycoproteins and beta-catenin, a subunit of the cadherin protein complex serving as a signal transducer among cells. They further demonstrated that hormonal treatment can up-regulate Rspo1 expression and hence established a method for *in vitro* expansion of MaSCs and maintenance of their undifferentiated status.

It is believed that this earlier work might benefit breast cancer research too, as it not only has unveiled the key role of hormones in MaSC expansion, but also established an economical method of MaSC *in vitro* culturing suitable for screening of hormone antagonists targeting breast cancers.

Both supported under the umbrella of the "CAS Strategic Priority Research Project on Stem Cells and Regenerative Medicine Research", the work published in *Genes & Development* and the current one in *Nature* mark the first results from ZENG's research since her joining SIBCB in 2010 as a "Hundred Talent Program" recruit. This talent program grants her with the funding needed to establish her own lab and conduct research at SIBCB. "I am thankful for the research environment, as I've found SIBCB is very good soil for seedlings," she remarks.

For more information please refer to:

Cai C, Yu QC, Jiang W, Liu W, Song W, Yu H, Zhang L, Yang Y, **Zeng Y. A**. (2014) "R-spondin1 is a novel hormone mediator for mammary stem cell self-renewal." *Genes Dev*. 28: 2205–2218.

Wang D*, Cai C*, Dong X, Yu QC, Zhang XO, Yang L, **Zeng Y. A**. (2014) Identification of multipotent mammary stem cells by protein C receptor expression. *Nature*. doi:10.1038/nature13851.

Zeng Y. A. and Nusse R. (2010) Wnt proteins serve as self-renewal factors for mammary stem cells and promote their long-term expansion in culture. *Cell Stem Cell*. 6(6): 568–77.