# 学　位　論　文
# （　要　約　）

Biological roles of SOX2 and mechanisms of regulation of its expression in human breast cancer cells

（ヒト乳がん細胞におけるSOX2遺伝子の役割とその発現調節機構）

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A tumor tissue is composed of different populations of tumor cells, in the cancer stem cell theory, which considers that only a small population of cells has the ability to initiate tumor and form the tumor cell heterogeneity. As cancer stem cell theory provides a promising way to cure cancer, it is important to identify and isolate cancer stem cells. In breast cancers, several genes are suggested as markers of breast cancer stem cells, but for now, there is still no standard marker of them.

SOX2 is an essential gene for embryogenesis and encodes a transcription factor. It regulates many downstream genes such as NANOG, LEFTY1 and FGF-4, all of which function in various stages of embryonic development. SOX2 is also well known as one of the essential transcriptional factors for generating inducing pluripotent stem cells and maintaining them; its expression level increases in a variety of cancers such as small cell lung cancer, gastric cancer, and prostate cancer and breast cancer. All the subtypes (luminal A/B, HER2 and triple negative) of breast cancer cells express SOX2 whereas normal adult mammary epithelial cells do not. These findings suggest that SOX2 expression is associated with the development of breast cancer and its malignant progression. However, the association remains unclear. In this study, I aimed to clarify the molecular mechanisms of the transactivation of SOX2 gene and the biological significance of SOX2 expression in human breast cancer cells.

As first step, I determined the essential transcriptional elements for SOX2 transactivation in breast cancer cells. Different from that in embryonic stem cells, SOX2 transcription of the breast cancer cells was dependent on its promoter but mot its enhancers SRRI or SRRII. And the promoter activities of SOX2 well correlated with endogenous expression levels of SOX2 among various breast cancer cell lines. These indicate the important role of SOX2 promoter region for SOX2 expression in breast cancer cells. Furthermore, the SOX2 promoter-driven fluorescent protein tdTomato vector were used to label and isolate cells with high SOX2 promoter activity. The fluorescent intensity reflected the promoter activity and SOX2 expression level. To know the biological characters of breast cancer cells with high SOX2 promoter activity, I established two sub-cloned cell lines from a single sorted cell with high SOX2 promoter activity. During this process of establishment of the sublines, I found that the cloned sublines with high SOX2 promoter activity could generate heterogeneous cells with different SOX2 promoter activities. Further cell biological analyses revealed that the breast cancer cells with positive SOX2 promoter

activity showed higher sphere-forming activity, a typical phenotype of CSCs, than those with low or no SOX2 promoter activity or parent cells. As several molecular markers of CSC-like cells are known in human breast cancer, such as CD44, ABCB1, ABCG2, NANOG, and TWIST1, I examined the expressions of these genes. RT-PCR analysis showed that the cells with high SOX2 promoter activity had high expressions of CD44, NANOG, C-MYC, ABCB1 and TWIST1 compared with those with its low or no activity. The expression level of CD24 of the cells with high SOX2 promoter activity was similar to that of the cells with low activity. These findings suggest that the cell population with high SOX2 promoter activity consists of a high concentration of CSC-like cells which are different from those already recognized in CSC population of breast cancer.

From these results, I understood that SOX2 was related to the cancer stem cell characters in breast cancer cells and that SOX2 promoter region was important for SOX2 expression.

As the next step, I aimed to find a minimal essential region for SOX2 promoter activity within the promoter region. A series of reporter vectors with deletion versions of promoter region were made and the most essential region was identified according to their activities in MCF-7 cells. After determining the essential region, I tried to identify the potential factors binding to this region by biotin-labeled pull down system and TOF-MS analysis. And three proteins were identified as the factor binding to the essential promoter region for SOX2 transactivation. Overexpression and knock-down of one (Factor X) of the three factors showed down- and up-regulated SOX2 expression levels, respectively, which indicated that the binding factor functioned as a repressor of SOX2 expression in breast cancer cells. Knockdown of the Factor X could increase the rate of SOX2 positive population in MCF-7.

To understand how the Factor X regulated SOX2 expression in breast cancers cells, I determined its binding site to the predicted potential element by CHIP assay. As the Factor X is known as a protein with several functional domains, I made domain-oriented deletion mutants and analyzed which domain(s) is important for its binding to SOX2 promoter region. And it was revealed that two domains located in relatively C-terminus were important for the repressor function. Further studies suggested that the Factor X suppressed SOX2 expression through preventing one of the members forming transcriptional machinery from binding to the essential promoter region. Finally, I investigated the role of the Factor X in the stemness of breast cancer cells.

The breast cancer cells of which the Factor X expression was inhibited formed increased numbers and sizes of mammnospheres, and this phenomenon was induced through the increase in expression of SOX2.

Different from ES cells or NSCs and other types of cancer, SOX2 expression in breast cancer is dependent on the activity of its promoter region, which has direct correlation with SOX2 transcription and mRNA expression level. The cells with high SOX2 promoter-detecting system can identify and isolate live cells with high SOX2 promoter activity, detailed analysis of the cells with high SOX2 promoter activity reveals that this population has CSC-like characters but with a different mRNA expression profile from other reported bCSCs. This system is useful for detecting and isolating the cell population with CSC-like feature in human breast cancer cells. Identified repressor represses SOX2 expression on mRNA and protein levels, knockdown of which can increase SOX2 expression and sphere-forming activity through regulating SOX2 and its downstream genes Deep investigation reveals that the repressor represses SOX2 expression in breast cancer cells through a unique way that it binds specific element in SOX2 promoter and prevent the important transcriptional factor from binding to SOX2 promoter region.

In conclusion, this study indicated that SOX2 expression in human breast cancer cells was dependent on its promoter region, which was different from that in embryonic stem cells. The novel SOX2 repressor identified in this study might disturb the binding of another indispensable factor forming transcriptional machinery to the SOX2 promoter region. And he repressor was thought to function as a regulator of stemness of human breast cancer cells through controlling the SOX2 expression. These suggested that regulatory system of SOX2 expression through this repressor played an important role in plasticity and/or maintenance of human breast cancer stemness.