

学位論文内容の要旨  
(Summary of dissertation)

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学位論文題名  
(Title of dissertation)

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)

**【Background and Objectives】**

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; It increases in a variety of cancers such as small cell lung cancer, gastric cancer, 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.

**【Methods and Results】**

First, to identify the cis-element essential for regulation of SOX2 expression in human breast cancer cells, I conducted a series of luciferase reporter assays, which revealed that POU-like binding element located on the SOX2 promoter region (-227 to -69) was one of the cis-elements essential for SOX2 promoter activity in human breast cancer cells. The assays also showed that SOX2 enhancer elements did not affect the SOX2 promoter activity. I next explored the proteins which bound to the cis-element essential for SOX2 expression. Pull down assay using biotinylated double-stranded DNA (SOX2 promoter region -227/-69) and LC-MS/MS analysis revealed that nuclear fraction of MCF-7 cells contained three kinds of proteins bound to the region: p54, eEF1 $\alpha$ 2 and hnRNPA1. Among them, p54 suppressed SOX2 expression in MCF-7 cells whereas two other proteins did not affect the expression; overexpression of p54 reduced promoter activity of p227, and inhibited p54 expression by sip54-transfection enhanced p227 promoter activity. ChIP assays proved that p54 directly bound to the region -227 to -69 of the SOX2 promoter, and that overexpression of p54 did not suppress the promoter activity of the P227 reporter harboring a mutation in the POU-like binding element. These findings indicated that p54 bound to POU binding-element on SOX2 promoter region (-227 to -69) and negatively regulated SOX2 expression.

Since p54 consists of several domains that are involved in RNA splicing, DNA transcription and formation of protein complex, next I investigated which domain(s) was important for the negative regulation of SOX2 expression by p54 in breast cancer cells. Reporter assays using deletion mutants of p54 revealed that the C-terminal region and the coiled-coil domain, which is reported to be a domain recognizing the POU binding-element, were indispensable for repressing p227 and the full length of SOX2 promoter activity. Further analyses by ChIP and immunoprecipitation revealed enhanced binding of pol II to p227 region by the inhibition of p54 expression and formation of a complex of p54 with pol II. From these results, I speculated that p54 disturbed the binding of pol II to p227 region through the formation of a complex with pol II, and resultantly inhibited SOX2 transcription. Finally, I examined whether SOX2 expression was involved in induction or maintenance of cancer stemness. I successfully isolated the cell population with high SOX2 promoter activity from MCF-7 cells by using reporter vectors with the fluorescent protein tdTomato gene that was driven by SOX2 promoter. MCF-7 cells with high SOX2 promoter activity showed high mammosphere-forming ability and expressions of some genes related to cancer stemness. And MCF-7 cells of which p54 expression was inhibited formed increased numbers and sizes of mammospheres. These findings suggested that the cell population with high SOX2 promoter activity consisted of a high concentration of CSC-like cells.

#### **【Discussion】**

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. p54 represses SOX2 expression on mRNA and protein levels, knockdown of p54 can increase SOX2 expression and sphere-forming activity. Profiling of sip54 demonstrates that knockdown of p54 also increases SOX2 downstream genes IGFBP5 and IL1R1 and activates PI3K signaling pathway. Deep investigation reveals that p54 represses SOX2 expression in breast cancer cells through a unique way that it binds to p227 region in SOX2 promoter by HMG-POU sequence and decreases SOX2 promoter activity by forming complex with pol II to disturb SOX2 transcription process.

#### **【Conclusion】**

In conclusion, this study indicated that SOX2 expression of human breast cancer cells was negatively regulated by p54 through its disturbance to the binding of Pol II to the SOX2 promoter region, and that the cell population with high SOX2 promoter activity contained cancer stem-like cells. These suggested that regulatory system of SOX2 expression through p54 repressor played an important role in plasticity and/or maintenance of human breast cancer stemness.