An E2F1-HOXB9 transcriptional circuit is associated with breast cancer progression

(E2F1 による HOXB9 転写制御と乳癌進展メカニズムの検討)

Thesis Summary

Homeobox B9 (HOXB9), a member of the homeobox gene family, is overexpressed in breast cancer and promotes tumor progression and metastasis by stimulating epithelial-to-mesenchymal transition and angiogenesis within the tumor microenvironment. HOXB9 activates the TGFβ-ATM axis, leading to checkpoint activation and DNA repair, which engenders radioresistance in breast cancer cells. Despite detailed reports of the role of HOXB9 in breast cancer, the factors that regulate HOXB9 transcription have not been extensively examined. Here I uncover an underlying mechanism that may suggest novel targeting strategies for breast cancer treatment.

To identify a transcription factor binding site (TFBS) in the HOXB9 promoter region, a dual luciferase reporter assay was conducted. Protein candidates that may directly attach to a TFBS of HOXB9 were examined by Q-PCR, electrophoretic mobility shift assay (EMSA), chromatin immunoprecipitation (ChIP), and mutation analysis.

A HOXB9 promoter region from -404 to -392 was identified as TFBS, and E2F1 was a potential binding candidate in this region. The induction of HOXB9 expression by E2F1 was observed by Q-PCR in several breast cancer cell lines overexpressing E2F1. The stimulatory effect of E2F1 on HOXB9 transcription and its ability to bind the TFBS were confirmed by luciferase, EMSA and ChIP assay. Immunohistochemical analysis of 139 breast cancer tissue samples revealed a significant correlation between E2F1 and HOXB9 expression (p<0.001). Furthermore, a CDK4/6 inhibitor suppressed E2F1 expression and also reduced expression of HOXB9 and its downstream
target genes.

The in vitro analysis identified the TFBS of the *HOXB9* promoter region and suggested that E2F1 is a direct regulator of *HOXB9* expression; these data support the strong correlation we found between E2F1 and HOXB9 in clinical breast cancer samples. These results suggest that targeting the E2F1/HOXB9 axis may be a novel strategy for the control or prevention of cancer progression and metastasis.