論文の内容の要旨
Dissertation Abstract

論文題目 **Functional Analysis of the Histone Methyltransferase Wolf-Hirschhorn Syndrome**
Dissertation Title **Candidate 1-like 1 (WHSC1L1) in Human Carcinogenesis**

（ヒト発がんにおけるヒストンメチルトランスフェラーゼ WHSC1L1 の機能解析）

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The emergence of effective cancer chemotherapy is one of the major medical advances of recent years. Adjuvant chemotherapy for lung, breast or colon cancer can augment the survival benefit afforded by surgical management. Even in patients with advanced solid tumors or recurrences after surgery, chemotherapy can offer lengthened survival of worthwhile quality. However the therapeutic approach for patients with recurrent tumor is limited, and its effects are usually partial and often disappointingly brief. In addition, most antitumor agents cause unexpected detrimental side effects. Therefore, it is critical to discover novel therapeutic targets to extend the capability of cancer chemotherapy and improve patient care.

DNA-histone complexes comprise the fundamental repeating unit of chromatin, and the multiplicity of histone modifications results in chromatin-dependent functions. This idea was previously proposed as the “Histone Code Hypothesis”. Histones, especially residues of the amino termini of histones H3 and H4 and the amino and carboxyl termini of histones H2A, H2B and H1, are susceptible to a variety of post-translational modifications such as phosphorylation, acetylation, methylation, ubiquitination, sumoylation, and glycosylation. Among histone modifications, methyl-lysine residues in nucleosomal histones are considered to mediate interactions with the macromolecular complexes that regulate chromatin-template processes such as transcription. Despite a large body of information for the prominent role of histone lysine methylation in transcriptional regulation, the involvement of their alterations in human diseases still remains unclear.

The aim of my research project is to clarify the importance of methyltransferase and demethylase in human carcinogenesis. As demonstrated in Figure 1, several histone-modifying enzymes have been shown to be deregulated in cancer cells. Among them, we had reported that SMYD3, a histone lysine methyltransferase, stimulated proliferation of cells and played an important role in human carcinogenesis through its methyltransferase activity.

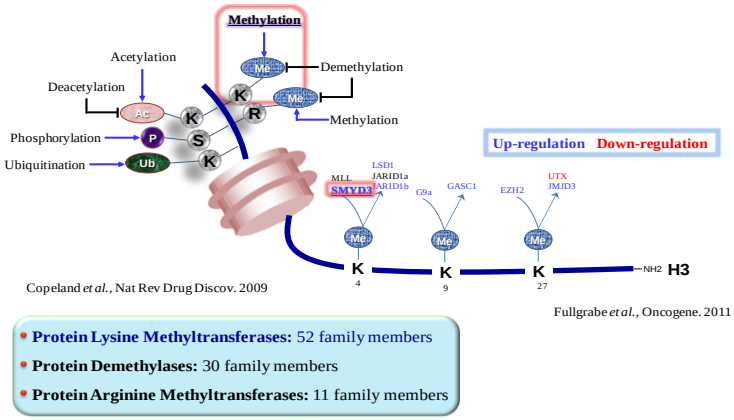

Figure 1. Histone-modifying enzymes deregulated in human cancer.

In order to investigate possible roles of histone lysine methyltransferases (HKMTases) in human carcinogenesis, I examined the expression profiles of human HKMTases in clinical tissues and found that expression levels of *WHSC1L1 (Wolf-Hirschhorn syndrome candidate—like 1)* were significantly up-regulated in various types of cancers compared with their corresponding normal tissues. *WHSC1L1*, also known as *NSD3* and *WHISTLE*, shows high sequence similarity to NSD2/WHSC1 (Wolf-Hirschhorn syndrome candidate-1) and NSD1, particularly in their C-terminal portion, which includes the SET domain responsible for the HMTase activity (Figure 2).

General Information
Cytogenetic band: 8p11.2
Protein size: 1437 amino acids; 138182 Da
Subcellular localization: Nuclear 69.6%, Plasma membrane 13.0%, Mitochondrial 8.7%, Cytoplasmic 4.3%, Vesicles of secretory system 4.3%
Conserved domain: PWWP, PHD, RING, AWS, SET, Post-SET
Function: Histone methyltransferase

Figure 2. Characteristics of *WHSC1L1*(*Wolf-Hirschhorn Syndrome Candidate 1-like 1).*

Although WHSC1L1 is known to function as a transcriptional regulator that mediates histone methylation, the biological function of the protein has not been well elucidated. Here, I demonstrated a possible involvement of WHSC1L1 in human cancers.

Firstly, expression levels of *WHSC1L1* transcript were significantly elevated in a various human cancers including bladder carcinoma and CML (Figure 3). Immunohistochemical analysis of bladder, lung and liver cancers confirmed overexpression of WHSC1L1 (Figure 4).

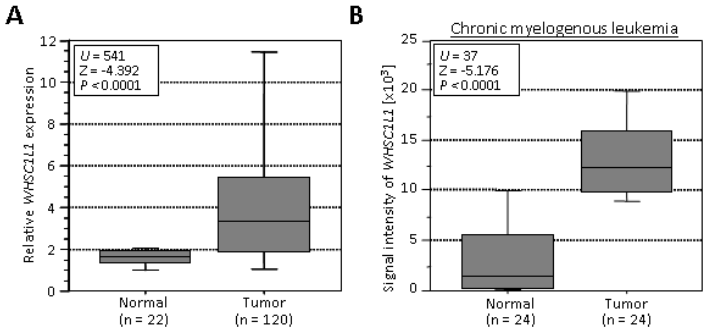

Figure 3. Elevated WHSC1L1 expression in human cancers. (A) Expression levels of *WHSC1L1* were analyzed by quantitative real-time PCR, and the result is shown by box-whisker plot (median 50% boxed). Relative mRNA expression shows the value normalized by *GAPDH* and *SDH* expressions. Mann-Whitney *U* test was used for statistical analysis. (B) Expression analysis of *WHSC1L1* in chronic myelogenous leukemia. Signal intensity of each sample was analyzed by cDNA microarray, and the result is shown by box-whisker plot (median 50% boxed). Mann-Whitney *U* test was used for the statistical analysis.

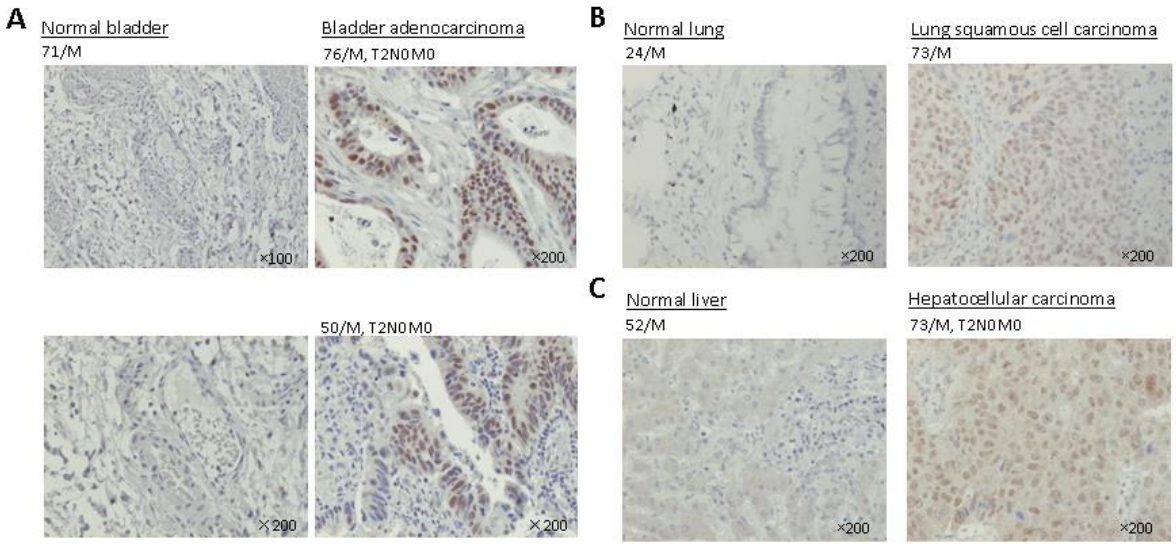

Figure 4. Tissue microarray images of clinical bladder tissues (A), lung tissues (B) and liver tissues (C) stained by standard immunohistochemistry for protein expression of WHSC1L1. Clinical information for each section is represented above histological pictures. All tissue samples were purchased from BioChain. Original magnification, x100 and x200.

Moreover, *WHSC1L1*-specific small interfering RNAs significantly knocked down its expression and resulted in the suppression of proliferation of bladder and lung cancer cell lines (Figure 5). WHSC1L1 knockdown induced cell cycle arrest at the G_2/M phase followed by multinucleation of cancer cells.

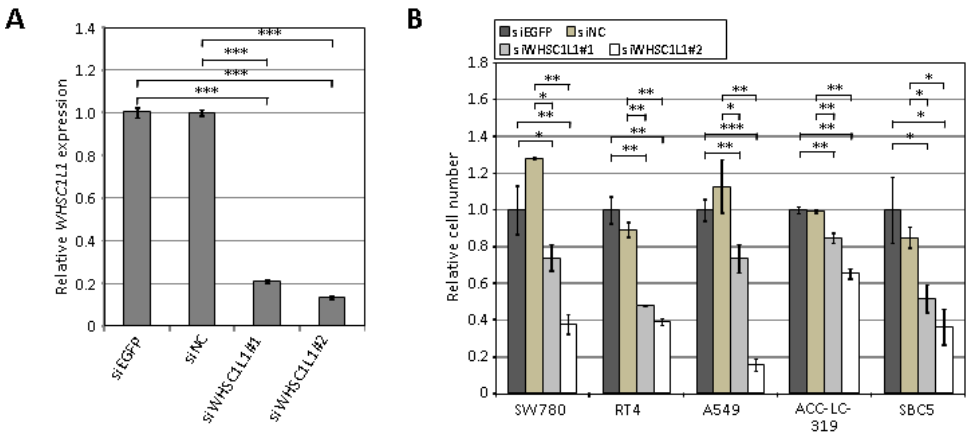

Figure 5. Involvement of WHSC1L1 in the growth of bladder and lung cancer cells. (A) Expression of *WHSC1L1* in A549 cells treated with two independent specific siRNAs targeting WHSC1L1 (siWHSC1L1#1, #2) was analyzed by quantitative real-time PCR. siRNAs targeting *EGFP* (siEGFP) and siNegative control (siNC) were used as controls. mRNA expression levels were normalized by *GAPDH* and *SDH* expressions, and values are relative to siEGFP (siEGFP = 1). Results are the mean ± SD of three independent experiments. *P* values were calculated using Student's *t*-test (***, $P < 0.001$). (B) Effects of WHSC1L1 siRNA knockdown on the viability of two bladder cancer cell line (SW780, RT4) and three lung cancer cell lines (A549, LC319 and SBC5). Relative cell number shows the value normalized to siEGFP-treated cells (siEGFP = 1). Results are the mean ± SD in three independent experiments. *P* values were calculated using Student's *t*-test (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

To further analyze the physiological function of WHSC1L1-signal pathway, I performed microarray expression analysis using Affymetrix's GeneChip® system. Messenger RNA from SW780 or A549 cancer cells were subjected to the analysis using HG-U133 Plus 2.0 Array. The expression profiles of these cells were compared with those of cells treated with control siRNAs (siEGFP and siFFLuc). As a result, WHSC1L1 was shown to affect the expression of a number of genes including *CCNG1* and *NEK7* those are known to play crucial roles in the cell cycle progression at mitosis. Since WHSC1L1 expression is significantly low in various normal tissues including vital organs, WHSC1L1 could be a good therapeutic target of various types of cancer.