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RESEARCH HIGHLIGHT

New insight into cancer therapeutics: Induction of differentiation by regulating the Musashi/Numb/Notch pathway

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The Musashi (Msi) family is a group of RNA-binding proteins characterized by two RNA recognition motifs (RRMs) and is evolutionarily conserved [1, 2]. In mammals, two isoforms of this family, Msi1 and Msi2, are co-expressed in neural precursor cells, including neural stem cells (NSCs). Msi2 exhibits high sequence homology with Msi1, which is more than 90% at the amino acid level within the RNA-binding domain. Msi2 is transcribed over the tissues ubiquitously [3] in contrast with Msi1 enrichment in neural stem cells or progenitor cells of the peri-ventricular area in the embryonic [4] and postnatal [5] mammalian brains. When both *Msi1* and *Msi2* genes were knocked-out, neurosphere formation was markedly inhibited; however, single knockout did not bring about such disturbance [6].

Originally, Msi was identified as a required factor for the asymmetric cell division of the sensory organ precursor cell (SOP) of the *Drosophila* adult external sensory organ [7]. In mammals, the function of Msi has been, thus far, found to activate Notch signaling through the translational repression of NUMB, which represses an intracellular Notch signaling, by binding to the 3′ untranslated region (UTR) of the *Numb* mRNA [8]. Activation of Notch signaling induces the transactivation of the promoter of the *Hes-1* gene (Figure 1). The activation of Notch-pathway is known to regulate the self-renewal of NSCs positively [9]. Previously, our laboratory reported that Msi1 and Msi2 developmentally controlled self-renewal of embryonic cells, as well as of adult CNS stem/progenitor cells [6]. Based on the evidence that tissue stem cells exist in many adult tissues, including the hematopoietic system, intestine, mammary gland, testis, skeletal muscle, skin, hair follicle and myocardium, other than the CNS and neural crest-derived tissue, Msi/Numb/Notch signaling is considered to be associated with many adult malignancies [2]. In fact, not only at the normal developmental stages, the Msi-signaling pathway is also reported to work during tumorigenesis in several adult tissues, including glioblastoma and esophageal, colon, pulmonary, mammary and bladder carcinomas [2, 10-13]. Intriguingly, in the case of esophageal adenocarcinoma, Msi1 expression was the highest in glands during early cancer development. In contrast, Msi1 level become weaker when esophageal adenocarcinoma grows into the advanced stage. Such findings have advocated a concept of "cancer stem cells" which expands to some kinds of solid tumors [10]. Then, Msi1 could be a versatile marker of "cancer stem cells" especially during an early phase with strong proliferative activities. This suggests the possibility that Msi1 might have some important roles as a trigger of proliferative switching from differentiated state to cancer production in a reverse direction to development.

Although a previous report showed that Msi2 gene was rearranged to form a *Msi2/HoxA9* fusion gene in a chronic myeloid leukemia (CML) case with the 7p15 breakpoint [14], the recent papers reported by Ito *et al*. [12] and Kharas *et al*. [13] were the first reports to show clearly the relationship between Msi2-inducing pathway and hematopoietic malignancy. These two reports suggested a new strategy for the therapy of aggressive leukemia. Using the *in vivo* models of CML in a chronic phase and in an aggressive blast phase [15], it was proposed that Msi2-Numb pathway should control the differentiation of CML cells and that this pathway could be the novel target of leukemia treatment [12].

Ito *et al*. first asked whether the levels of MSI/NUMB expression and Notch signaling altered in the CML model mice [12]. Myeloid blast crisis was modeled by transplanting hematopoietic stem cells (HSCs)-enriched KLS cells,

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which was transduced with *Bcr-Abl* and *Nup98-HoxA9*. HOXA9 regulates expression of many genes that are implicated in hematopoiesis and in tumorigenesis. Using the established mouse model representing chronic phase and myeloid blast crisis CML (initiated by the *Bcr-Abl* translocation and progressed by *Nup98-HoxA9* expression) [16], they found *Numb* was expressed at significantly lower levels and *Msi2* was particularly elevated in the blast crisis phase. Notably, *Msi2* was expressed at higher levels than *Msi1* in the CML mice model, especially in hematopoietic stem cell-enriched populations including KLS cells and the lineage-negative fraction of blast crisis CML. Moreover, they identified a putative HoxA9-binding element at the position –5.7 kb in the murine *Msi2* gene. *Nup98-HoxA9* transduced with retroviral vector along with *Bcr-Abl* could overexpress the *Msi2* gene in KLS cells and upregulation of MSI2 expression led to downregulation of NUMB (Figure 1). At the same time, Kharas *et al*. established doxycycline-inducible Msi2 expression systems both *in vitro* and *in vivo*, which culminated in hematopoietic colony-forming with more immature myeloid phenotype *in vitro* and expansion of hematopoietic stem cells (HSCs) and short-term progenitor cells *in vivo* [13]. They also transduced doxycycline-inducible Msi2 cells with the *Bcr-Abl* oncogene and transplanted them into mice. Consistent with the other report, Msi2 induced immature myeloid leukemia that mimicked CML blastic crisis phase. Conversely, when Msi2 was knocked down, an increased amount of NUMB protein in the LAMA-84 cell lines, derived from the blast crisis CML patient, was observed with immunoblot.

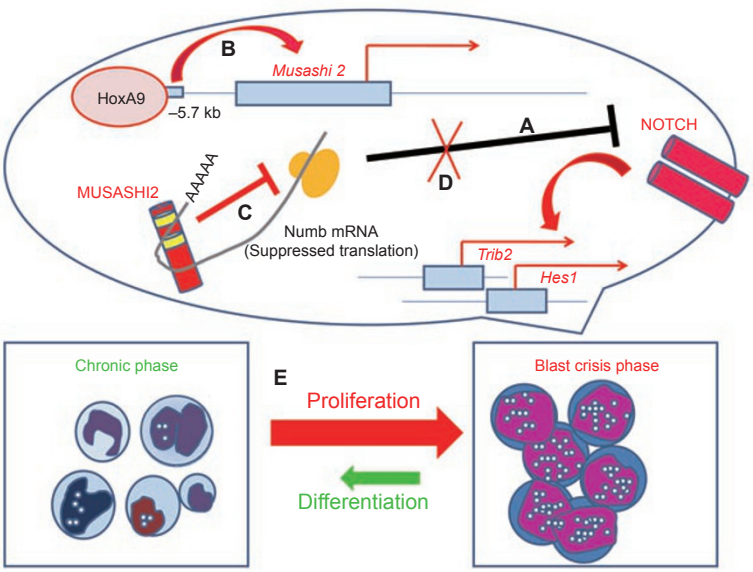

Figure 1 Scheme of the CML state in an aggressive blast crisis phase. In chronic phase, Numb blocks activation of the Notch signaling (A). During human CML progression, the following changes occurs; *Nup98-HoxA9* oncogene binds the putative element at 5.7 kb upstream of transcription start site to trigger the upregulated expression of *Musashi2* gene (B). The elevated level of MUSASHI2 leads to the downregulation of NUMB, by binding to the 3′ UTR of *Numb* mRNA to inhibit translation (C). Inhibition of Notch signaling is cancelled by suppression of NUMB (D), leading to the elevated expression of the targets, *Hes1* gene and *Trib2* gene. A series of these activation cause proliferative change from chronic phase to blast crisis phase of CML (E). Red symbols and letters represent increased level of each factor and enhanced activation (or inhibition) in blast crisis phase CML.

Then, Ito *et al*. asked whether human leukemia in blast crisis showed aberrant up-regulation of Msi2 as well. Analysis of samples from 30 Korean and British patients showed significant higher levels of *Msi2* gene expression in blast crisis CML. As predicted from the CML mice model, microarray data of 90 samples from banks in the United States showed elevated levels of Msi2, HoxA9 and Hes1 and decrement of Numb among most of CML patients in the blast crisis phase. Similarly, Kharas *et al*. [13] showed the increased level of Msi2 and decreased level of Numb from 33 samples of myeloid blast crisis by comparing with 57 samples of chronic phase CML obtained in the United States.

It is particularly noteworthy that Ito *et al*. suggested the apparent clues suppressing tumor growth and mortality rates in the established CML model mice *in vivo*. They showed that a higher Numb expression made leukemia more differentiated and unable to propagate disease markedly. In fact, transplantation of cells expressing Numb decreased the frequency of developing leukemia in the model mice, from 83% to 63%. When the authors transplanted cells transfected with Numb, from primary transplanted mice for donor-derived cells, into irradiated recipient secondary mice, the survival rate to evaluate propagation of disease was elevated compared to the control, from 20% to 93%. These results showed that the elevated levels of NUMB can inhibit disease progression. Additionally, using gene-trap mutants or short hairpin RNA approach, loss of Msi2 impaired leukemia growth and increased survival rates significantly *in vivo*, especially in blast crisis CML. Similar to NUMB induction, Msi2-shRNA induced the differentiation of leukemic cells and inhibited their propagating ability [12].

Ito *et al*. also reported the association of Msi2 with high risk of relapse after allogeneic transplantation and poorer outcomes, indicating the possibility to make use of MSI2 as an early marker of advanced CML. On the other hand, Kharas *et al*. confirmed that *Msi2* expression had relevance to poor clinical prognosis among at least congenitally normal AML subjects. Moreover, among them, they showed that the population, whose gene signatures were negatively correlated with genes altered by the Msi2-knockdown, was associated with poor prognosis. Using multivariate analyses with numbers of Msi2-associated factors, we might predict the prognosis or definitive diagnosis more accurately.

Novel findings about the Msi2/Numb/Notch signaling pathway described above will shed light on a key to understanding development of hematologic malignancies including blast crisis phase CML. Other recent papers have revealed the relationship between Notch signaling/Hes1 and CML progression [17]. Together with these findings, each of Msi family could be a key regulator of several intracellular pathways, deciding self-renewal of the stem cells, tumorigenesis and differentiation, which is cell-fate exactly.

Further studies about relationship between Msi-signaling pathway and malignancies will be investigated. Then, differentiation induction into mature asymmetric cells by modulating the pathway in cancer cells is expected to be the novel therapeutics for advanced leukemia and other solid carcinomas.

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