Synopsis of Original Research Paper

Generation of a novel system for functional analysis of genes involved in growth, differentiation, and cell death in epidermal cells

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Somatic cells from patients with Bloom (Blm) syndrome show an increased rate of homologous recombination and therefore, results in generation of bi-allelic mutation from mono-allelic mutation. We have used a tetracycline-regulated Blm allele (Blm^{tet}) to introduce bi-allelic mutations in ES cells (5). Phenotype-based genetics is now achievable and raises new possibilities for identifying novel gene functions.

Although we would like to apply this system in mice or their somatic cells, addition of doxycyline (dox), an analogue of tetracycline, to Blm^{tet} mice did not result in sufficient reduction of Blm expression because of leaky Blm expression in vivo. This leaky expression has been markedly reduced in vitro by using a tetracycline-controlled trans-silencer. A new tetracycline cassette containing the trans-silencer gene was constructed and inserted into the Blm gene to generate a new Blm allele ($Blm^{tet\text{-}new}$) in ES cells. We tested the dox-dependent regulation of Blm expression and possible introduction of bi-allelic mutations in mice or their somatic cells derived from the ES cell line bearing $Blm^{tet\text{-}new}$ and found that sister chromatid exchange (SCE), a hallmark of Bloom gene deficiency, was markedly increased in lymphocytes derived from mice bearing $Blm^{tet\text{-}new}$ alleles, suggesting that efficient bi-allelic mutations have been introduced in mice.

We reported that Sleepng Beauty transposon (SB) jumps efficiently in mouse genome (1) and SB-mediated mutagenesis is possible (3). Moreover, we have been recently reported potential of SB for comprehensive mutagenesis (6). Since the SB transposon system has a unique feature that can introduce various mutations in somatic tissues, this allows us to search for many novel gene functions in a single mouse.

Combination of the two systems described above opens a new research field for "**somatic cell genetic biology**". In this system, SB-mediated transpositions cause many mono-allelic gene disruptions in somatic cells followed by the introduction of bi-allelic mutations from them.