P1:103

Studies of Osteosarcoma Metastasis Driver Genes using Transposon Mutagenesis in Mice and TALEN-Mediated Gene Knockouts in Osteosarcoma Cell Lines

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Background: Random insertion of Sleeping Beauty (SB) transposons in somatic cells produces mutations leading to tumor development, which has successfully identified candidate genes for many types of cancer.

Methods: We created transgenic mice that develop osteosarcoma (OS) via SB transposon-mediated mutagenesis activated by an osteoblast specific Osterix-cre recombinase transgene (Osx-Cre). Tumorigenesis was accelerated with a Trp53 pathway deficient background (Trp53R270H/+). Ligation-mediated PCR, next generation DNA sequencing, and TAPDANCE software identified recurrent transposon sites in DNA isolated from primary tumors and metastatic nodules. Using transposon integration sites as molecular tags, non-supervised hierarchical clustering analysis assessed relatedness among metastatic and primary tumor sets. Copy number variation of candidate genes was evaluated in human matched normal, primary and metastases OS samples.

Results: Our quadruple transgenic mice (Osx-Cre; R26-LSL-SB11; LSL-Trp53R270H; T2/Onc) develop OS with an average latency of 10.5 months and a penetrance of 75% (n=96), compared to 17 months and 60% (n=49) in Trp53R270H/+ controls. SB tumors resemble human OS in gross anatomy, histological appearance, and presence of collagen. Over 100 metastatic nodules were collected from 16 quadruple transgenic mice. Analysis of recurrent SB integration sites revealed the well-known OS genes RB1 and CMYC in primary tumors, validating the screen, and novel genes not previously reported, including 10 genes common among metastatic nodules. Metastases from the same mice were clonal derivatives from the primary tumor and generally more related to each other than to the primary tumor, even when collected from different organs. Many of the genes identified by the SB screen mapped to regions with copy number changes in human OS tumors.

Conclusion: The SB screen revealed high clonality among metastases and identified several candidate metastatic drivers. Functional validation using published in vitro assays for migration and invasion is being conducted on cell lines derived from lung and primary tumor pairs collected from the SB mice and well-characterized human and murine OS cell lines: U2OS, HOS, MNNG/HOS, 143B, K12, and K7M2. Gene expression will be increased using over expression vectors containing cDNA and/ or silenced using transcription activator-like effector nuclease (TALENs) mediated gene knockout.

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