Cancer-associated chromosomal deletions: Size makes a difference

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Latest technological advances have empowered researchers to systematically examine the cancer genome at unprecedented resolution. Recent studies revealed that somatically acquired loss-of-heterozygositys (LOHs) are extremely common in human cancer.\textsuperscript{1,2} In a typical cancer sample, about 25% of the genome is affected by chromosome arm-level deletions and 10% by focal deletions, with about 2\% overlap.\textsuperscript{1} Although recurrent large-scale LOHs are more frequently observed in human cancer samples, it still remains mainly unclear how these chromosomal alterations contribute to cancer development and progression. It is well established that deletions of tumor suppressor genes or non-coding RNAs could confer selective growth advantage to the cancer cells. However, chromosome-arm LOHs lead to a concomitant loss of hundreds of protein-coding genes, non-coding RNAs, and regulatory elements, making it challenging to predict the functional outcome of large-scale chromosomal aberrations.

The short arm of chromosome 8 (8p) is one of the most recurrently deleted genomic regions in the majority of human epithelial cancers.\textsuperscript{3} Loss of 8p is detected at early stages of breast cancer development and associated with poorer patient survival, strongly suggesting a role for 8p LOH in tumor initiation and progression. Multiple functional studies verified the tumor suppressive properties of about 20 genes located in the 8p region. These functional studies clearly demonstrated that suppression of individual 8p-located genes can provide growth advantage and/or promote tumor growth. However, none of the 8p-located genes fulfills the Knudson’s 2-hit criteria for tumor suppressor genes,\textsuperscript{4} raising the possibility that some recurrent cancer-associated LOHs may provide a selective advantage by simultaneously targeting multiple hits. In fact, a loss-of-function screen revealed that multiple genes on chromosome 8p can cooperatively inhibit tumorogenesis in mice, and that their co-suppression can synergistically promote tumor growth.\textsuperscript{5}

On the other hand, the deleted regions contain not only STOP genes, which negatively regulate cell growth, but also GO genes, which are either essential genes, or genes that promote cell growth. STOP and GO genes were identified by functional shRNA screens using human mammary epithelial cells and a set of cancer cell lines.\textsuperscript{5} The gene expression analysis of The Cancer Genome Atlas (TCGA) breast cancer patients revealed that loss of 8p results in down-regulation of 5 STOP genes and 5 GO genes (Fig. 1). In concordance with the idea that STOP genes might represent tumor suppressor genes, several functional studies reported that suppression of 8p-located STOP genes, MTUS1 or TUCS3, enhances cell proliferation and tumor growth. On the other hand, 8p-located GO genes, PTK2B and CLU, are known to promote cell growth and have been implicated in the progression of several types of epithelial cancer.\textsuperscript{4} Hence, a positive impact on cell growth due to the cumulative reduction in the dosage of 8p-located STOP genes could be balanced by deleterious effects caused by reduced dosage of GO genes. Therefore, novel approaches integrating the complexity of copy number aberrations in tumors should be more appropriate in elucidating the functional outcome of large-scale chromosomal deletions.

Using TALEN-based chromosomal engineering technology, we created clinically relevant cell models that mimic 8p LOH commonly observed in breast cancer patients.\textsuperscript{3} In particular, we introduced a targeted 8p LOH into non-malignant human mammary epithelial MCF10A cells. We confirmed that gene expression profiles of 8p-deleted MCF10A cells are significantly overlap with gene expression profiles of breast cancer patients harboring 8p LOH, indicating our genetically engineered human cells represent a relevant model to elucidate tumorigenic phenotypes mediated by 8p LOH. Although multiple 8p genes have been implicated in tumor suppression, loss of 8p 2Mb-35Mb region does not confer tumorigenic transformation. Moreover, 8p LOH does not enhance cell transformation triggered by ERBB2, MYC, or p53 depletion. This result strongly suggests that the ratio of STOP and GO genes affected by chromosomal loss could...
be a key determinant for the functional outcome of the deletion. Consistent with this idea, we found that LOH of 8p 22-30Mb region, which leads to downregulation of 3 GO genes and only 1 STOP gene, results in a significant growth inhibition. These data also imply that large-scale aberrations, which encompass hundreds of genes, may create vulnerabilities that fundamentally differ from those that arise due to a loss of a single gene.

Further systematic analysis of cells with 8p LOH revealed that loss of 8p confers a higher resistance of 8p-deleted cells to hypoxic stress due to up-regulation of ceramide-induced pro-survival autophagy, providing growth advantage at early stages of tumor development. 8p-deleted cells are also highly resistant to taxane-based chemotherapy. Moreover, upregulation of the cholesterol and fatty acid metabolism pathways triggered by loss of several 8p genes leads to increased invasiveness and intravasation. This could explain the poorer survival rates of breast cancer patients harboring 8p LOH.3

In summary, our study confirmed that large-scale chromosomal alterations should be studied as distinct genomic events. Taking into account the extent and complexity of chromosomal deletion, TALEN- or CRISPR/Cas9-based chromosomal engineering is the most appropriate approach to elucidate the contribution of large-scale chromosomal aberrations to tumorigenic transformation. Furthermore, the generation of visibly marked deletions will provide essential reagents for maximizing the efficiency of large-scale functional genomics efforts and accelerate the functional annotation of the human cancer genome.

Disclosure of potential conflicts of interest
No potential conflicts of interest were disclosed.

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