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Letter to the editor

Whole genome loss of heterozygosity profiling on oral squamous cell carcinoma by high-density single nucleotide polymorphic allele (SNP) array

Most human cancers are characterized by genetic instabilities [1]. Chromosomal aberrations include segments of allelic imbalance identifiable by loss of heterozygosity (LOH) at polymorphic loci, which may be used to implicate regions harboring tumor suppressor genes [2–4]. LOH patterns can be generated through allelotyping using polymorphic microsatellite markers; however, due to the limited number of available microsatellite markers, the rather tedious and labor-intensive procedure, and the requirement for large amounts of DNA, only a modest number of microsatellite makers can be screened. High-density whole genome allelotyping cannot be performed. Single nucleotide polymorphisms (SNPs) offer many advantages for genetic analysis, including their prevalence in the genome and the ease of assay. The unique advantage of SNPs for LOH analysis is that, unlike microsatellites, they are not susceptible to the repeat expansion that is so often observed in cancer [5–7] and thus provide higher-fidelity markers for tracking the fate of chromosome segments. SNP-based LOH analysis is therefore the most logical approach for genome-wide allelic imbalance profiling. Recently, several studies have successfully utilized the Affymetrix (Santa Clara, CA) HuSNP GeneChip, which contains 1494 SNPs for identifying consistent LOH regions in small cell lung, breast, bladder, and prostate cancer [8–12].

We present the feasibility of using the newly developed Affymetrix 10K SNP Mapping Array for genome-wide LOH profiling by genotyping of 11 human oral epithelial cell lines (Fig. 1). This new SNP array contains a large collection of SNPs (more than 10,000 SNP alleles) with high frequencies of heterozygosis (36.4% heterozygosity for the general population) and provides a high resolution coverage of the whole genome. The genomic DNAs were isolated from cultured squamous cell cancer (SCC) cell lines using the Qiagen (Valencia, CA) genomic DNA isolation kit. The labeling, hybridization, washing, and staining of the 10K SNP mapping array was performed according to the standard Single Primer GeneChip Mapping Assay protocol (Affymetrix). The SNP genotype calls were generated using the Affymetrix Genotyping Tools (GTC) software. The LOH maps for these cells were generated using the novel informatics platform,

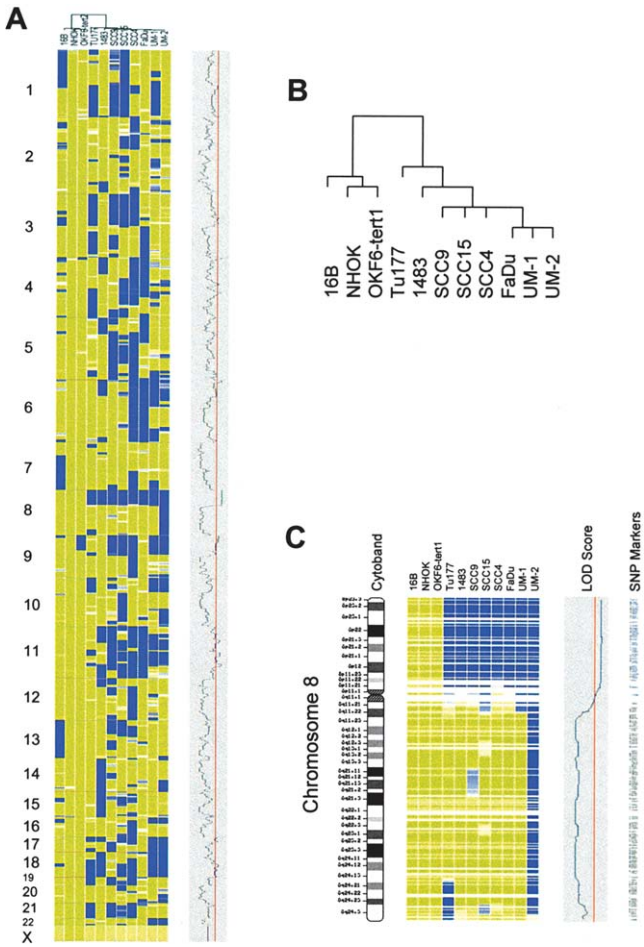

Fig. 1. SNP array-based LOH maps of oral cancer cell lines. (*A*) The LOH regions were detected and demarcated using dChipSNP [13]. Each column represents one cell line, and each row represents a SNP marker. Blue, LOH; yellow, retained; gray, uninformative; white, no call. (*B*) Hierarchical clustering of cell lines based on LOH similarity. (*C*) Magnified view of the LOH map for chromosome 8. The blue curve in the shaded gray box denotes the logarithm of the odds (LOD) score, representing the excessive sharing of LOH. The LOD = 2.0 threshold is indicated as a red line. Along the far right, the position and name of each SNP are shown. Where the density of SNPs was too high to allow a full display, hidden SNPs were indicated by small blue dots.

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dChipSNP [13]. Briefly, a hidden Markov model is used to infer LOH status from SNP calls of each sample, and a LOD (Logarithm of the odds) score for LOH is computed [14] to evaluate the likelihood that a particular locus harbors a caner-related gene. Hierarchical clustering based on LOH calls in the specific regions with LOD score exceeding particular threshold was carried out as described previously [15].

As shown in Fig. 1A, NHOK, the normal keratinocyte cell line, has no LOH across the genome. The oral tumor cell lines Tu177, 1483, SCC9, SCC15, SCC4, FaDu, UM-1, and UM-2 and the immortalized but nontumorigenic cell lines 16B and OKF6-tert1 have various LOH regions. Hierarchical clustering of cell lines based on LOH similarity showed two major groups. The first group contained 16B, NHOK and OKF6-tert1; a second group contained all eight tumor cell lines (Fig. 1B). Chromosome arm 8p has been identified as a frequent LOH region, where all 8 tumor cell lines (but not NHOK, 16B, or OKF6-tert1) exhibited LOH (Fig. 1C). This is in agreement with published findings using microsatellite-based LOH approaches [16–19].

Our results show the feasibility of using the Affymetrix 10K SNP mapping array for genome-wide LOH study on oral SCC cell lines. Furthermore, our data yield important information on consistent LOH patterns in oral SCC, which implicate regions harboring tumor suppressor genes. The identification of these candidate genes will facilitate the understanding of tumorigenesis of oral cancer. Further studies are needed to functionally evaluate those candidate genes in the consistent LOH regions.

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