Abstract

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Project Title: Two Hits Hypothesis Study in Nasopharyngeal Carcinoma

Investigator: Dr. Apiwat Mutirangura

Email Address: mapiwat@chula.ac.th

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The main objective of this study was to determine the precise frequency of chromosome 14q and 13q loss of heterozygosity in nasopharyngeal carcinomas and to define its minimal deletion regions. Regarding chromosome 14q, thirty-nine tumors were selected for PCR-based deletion mapping using 19 microsatellite polymorphic markers spanning the long arm of this chromosome, Loss of heterozygosity for at least one marker was observed in 29 (74.4%) tumors, while 24 of these tumors displayed partial loss and provided an informative basis for detailed deletion mapping. Three minimal regions of loss were delineated, the first defined by markers D14S278 and D14S288, the second being between DI4S51 and the telomere. These data confirmed 2 potential tumor-suppressor-gene loci at 14q12-13 and 14 q32. Interestingly, the third region of loss was located at the T-cell-receptor delta chain locus. This may reflect another tumor-suppressor-gene locus at 14q11.2, or may be the consequence of a specific genomic rearrangement of this region. In addition, these allelic losses occurred with high frequency in all tumor grades and stages and in all histological sub-types. To identify tumor suppressor gene loci on chromosome 13 responsible for nasopharyngeal cancer (NPC) development, we analyzed loss of heterozygosity (LOH) and RE protein expression in paraffin embedded tissues. Normal and tumor DNA were extracted from microdissected samples, and their whole genomes were amplified using degenerate oligonucleotide primers. The polymerase chain reaction (PCR) products were analyzed by repeated amplification using primers derived from 16 microsatellite regions spanning the long arm of this chromosome. Among 50 informative cases, LOH was observed in 44 tumors. Thirty-one tumors displayed partial loss and provided an informative basis for detailed deletion mapping. Three minimal regions of loss were delineated; the first flanked by D13S120 and

D13S119, and the third by D13S137 and 13qter, These 3 regions were linked to BRCA2 on 13q12, RBI on 13q14, and 13q14.3-ter, respectively. Seven and 4 cases showed LOH either on 13q12 or 13q14, respectively. Nineteen cases showed LOH of both loci separately. One NPC displayed 13q12 and 13q14.3-ter LOH. RE protein expression was detectable in 76% of the cases. Ten out of 15 cases with the allelic losses limited to 13q14 showed RE protein expression. Contrasting that. 6 out of 7 cases devoid of RE protein expressions showed 13q14LOH. In conclusion, 13qLOH. involving 3 tumor suppressor gene loci, appears to be a frequent genetic event occurring during NPC development. However, other tumor suppressor genes besides RBI. may be responsible for the majority of 13q14LOH. These findings suggest that the genetic alteration of chromosome 13 and 14 is common and crucial during nasopharyngeal-carcinoma development.

Since information from 14qLOH study suggested there is a specific mechanism involve LOH at T cell receptor delta gene, this study looked for supportive evidence if erroneous V(D)J recombination might serve as a mechanism responsible for EBV-associated NPC development. The result of this part is a preliminary data for studying in a TRF basic research grant topic molecular genetics of NPC development from the same principle investigator. Thus the data will not present in this report but will discuss during the oral presentation and the detail will be included in another report.

Keywords: Nasopharyngeal Carcinoma, Loss of Heterozygosity, Epstein-Barr Virus, Two Hits Hypothesis