Immortalization of Normal Human Nasopharyngeal Epithelial Cells

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Nasopharyngeal carcinoma is a common cancer in Hong Kong. Epstein-Barr Virus (EBV), a ubiquitous human herpesvirus, is associated with the development of malignancies, such as carcinomas of the nasopharynx and lymphomas, in both epithelial and lymphoid cells. The mechanisms responsible for oncogenic transformation of EBV-infected cells are incompletely understood. Up to now, most of the functional studies on the transformation ability of EBV were demonstrated in either non-nasopharyngeal epithelial or transformed cell line, which may not reflect the actual cellular signaling in normal nasopharyngeal epithelial cells *in vivo*. Hence, a normal human nasopharyngeal epithelial cell line would need to be generated for further investigation of the functions of EBV latent genes.

We have stably expressed telomerase and a cyclin-dependent kinase 4 mutant ($Cdk4^{R24C}$) in a normal human nasopharyngeal epithelial primary culture in order to immortalize the cells. Telomerase is a ribonucleoprotein that maintains the telomeric length. Expression of telomerase extends the lifespan of human fibroblast with a normal karyotype. $Cdk4^{R24C}$ is a mutant that is insensitive to the cell cycle inhibitor $p16^{INK4a}$. Our preliminary results showed that stable expression of telomerase and $Cdk4^{R24C}$ increased the passage number of proliferation of a normal human nasopharyngeal epithelial primary culture.

We are also expressing other genes such as Id1 (Inhibitor of differentiation 1) and a dominant negative mutant of p53 (p53DD) in normal human nasopharyngeal epithelial cells, as well as culturing the cells on a feeder layer, to study whether these approaches could extend the lifespan of the cells.