109 **The role of non-homologous end-joining in oral carcinogenesis**

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DNA DSB repair system consists of homologous recombination (HR) and non-homologous end-joining (NHEJ). In humans, NHEJ is the predominant repair system. The DNA double strand break repair genes are important caretakers of genome stability and suggested to play a role in the development of human carcinogenesis. However, no evidence has been provided showing that these genes, such as *Ku70, Ku80* and *XRCC4,* were associated with oral oncology. In this hospital-based case-control study, the associations of *Ku70, Ku80* and *XRCC4* polymorphisms with oral cancer risk in a Taiwanese population were firstly investigated. As for *Ku70*, the genotypes of promoter T-991C (rs5751129), promoter G-57C (rs2267437), promoter A-31G (rs132770), and Intron3 (rs132774) were determined by PCR-RFLP method. As for *Ku80*, the genotypes of promoter G-1401T (rs828907), promoter C-319T (rs11685387), and Intron19 (rs9288518) were determined. As for *XRCC4*, the genotypes of C-1622T (rs7727691), G-1394T (rs6869366), G-652T (rs2075685), C-571T (rs2075686), Intron3 DIP (rs28360071), codon 247 (rs3734091), Intron7 DIP (rs28360317) and Intron 7 (rs1805377) were determined. Four out of all the single nucleotide polymorphic genotypes we investigated (T-991C of *Ku70*, G-1401T of *Ku80*, Intron3 and codon 247 of *XRCC4*), were found to be significantly associated with oral cancer susceptibility, based on the evidence from genotypic and allelic distribution analysis between the oral cancer patient and control groups. To sum up, we have firstly found that there are several novel biomarkers of oral cancer in Taiwan located on the NHEJ genes, *Ku 70, Ku 80* and *XRCC4*, and they may be useful biomarkers for primary prevention and anticancer intervention.

110 **Optimization of antiviral RNAi molecules: a Dicer-independent shRNA processing route**

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A gene therapy based on RNA interference (RNAi) has been proposed as a promising and durable antiviral approach against HIV-1 infection. RNAi is induced by small interfering RNAs (siRNAs) that can be processed from vector-encoded short hairpin RNAs (shRNAs) by the Dicer endonuclease. One strand of the siRNA duplex subsequently instructs the RNA-induced silencing complex (RISC) with the catalytic AGO2 protein to mediate cleavage of the complementary HIV-1 RNA target.

Although a single shRNA can potently suppress viral replication, HIV-1 escape mutants will eventually emerge that contain a mutation or deletion in the siRNA target sequence. We demonstrated that a combinatorial approach by expressing multiple shRNAs can durably inhibit HIV-1 and prevent viral escape. However, RNAi may also cause serious side-effects, which is siRNA-sequence and dose-dependent. Thus, there is a need to select potent shRNAs that are properly processed to reduce the required shRNA concentration in order to avoid toxicity. To date only a few shRNA variations were tested.

We tested a set of shRNAs that differ in sequence and structure of the hairpin loop. In addition, we tested shRNAs with varying hairpin stem lengths. We found that the sequence of the shRNA loop can significantly affect the shRNA activity and the strand selection process. We described a novel shRNA loop design that provides universal improvement, independent of the specific shRNA and the cell type.

Further analyses indicated that some shRNAs with minimized stem length result in the generation of RNA fragments that are not characteristic of standard Dicer-processing products, suggesting the involvement of an alternative processing route. Characterisation of the RISC-associated small RNAs revealed the exact nature of this route.

111 **The PAX6 SWITCHBOARD: Conserved noncoding elements acting as regulatory SWITCHES**

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PAX6 is considered the 'Master regulator of eye development' and mutations leading to altered PAX6 expression are a major cause of the congenital eye malformation aniridia in humans. Aniridia cases with breakpoints outside the PAX6 coding region have led to the identification of several highly conserved tissue-specific noncoding elements (CNEs). The role of these elements in regulating the complex spatio-temporal expression pattern of PAX6 is well established. Here we describe our attempts towards the identification of trans-acting factors which modulate PAX6 expression by controlling the activity of PAX6 CNEs. We have been using a combination of mouse and zebrafish reporter transgenics to assess the effects of mutating predicted TFBS on reporter gene expression patterns driven by specific CNEs. Initial efforts have focussed on the SIMO lens enhancer, located 150kb downstream of Pax6, beyond the most distal aniridia patient breakpoint. Mutant SIMO elements were made bearing point mutations in predicted TFBS (PAX6, HMX, ETS and MEIS). Severe loss of lens expression is seen in mutants for putative PAX6 and HMX/Nkx5.3 binding sites, while MEIS binding site mutants showed gain of reporter gene expression in the retina. We have validated these results by morpholino knockdown of pax6, hmx1 and meis-1 in SIMO-WT-stable zebrafish reporter transgenics. This approach allows a quick screen for upstream regulators of PAX6, acting via the CNEs to control PAX6 expression. The method holds the promise of enabling quick screening of patient point mutations identified in CNEs for their relevance in precisely modulating protein expression, thereby leading to disease.

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Withdrawn