CHAPTER 6

General Discussion

No matter how dark a night
It must be followed by dawn...

No matter how painful the fight
Cancer must go so you can live on...

Image adapted from yurtopic.com
The study aims at understanding the role of the candidate genes RBSP3, LIMD1 and CDC25A in the development of squamous cell carcinomas of head and neck. Previous studies formulating the progression model of HNSCC had indicated the association of several genes including the candidate genes in the development of pre-malignant lesions of head and neck. However, the studies were performed in separate cohorts, thereby negating the cumulative effect of alterations of the candidate genes during the development of HNSCC. Thus, in order to study the association of the genes in pre-malignant and malignant head and neck lesions, our study was performed in the same group of samples. The profile of the candidate genes was compared to the stem-cell like basal layer for all analysis to understand how the profile alters during tumorigenesis.

Characterization of the molecular signature of RBSP3, LIMD1 and CDC25A in normal oral epithelium, along with their alterations during tumorigenesis and response to neoadjuvant chemotherapy indicates the importance of the candidate genes in the development of HNSCC. Although the candidate genes play the role of tumour suppressors, their mode of regulation is different. RBSP3 maintains its low expression/high promoter methylation signature of the basal layer of normal tissues during tumorigenesis through additional deletion with modest gain of its expression in tumours subjected to neoadjuvant chemotherapy (Figure 6.1). This might be due to reversal of methylation of the promoter of RBSP3 due to effect of the drugs used in therapy, leading to its gain of expression in post-treated tumours. Conversely, the high expression/low methylation basal signature of LIMD1 is radically altered through enhanced methylation/deletion with regain of its expression in HNSCC post NACT. Similarly, CDC25A loses its high basal expression signature though deletion with maintenance of similar expression in tumours post NACT (Figure 6.1). Moreover, etiological factors tobacco and HPV were important for regulation alterations of the genes and for determining the overall survival of HNSCC patients.

Thus, the fine balance between proliferation/differentiation in the stem cell like basal layer undergoes modification during development of HNSCC, as well as when the tumour is subjected to therapeutic intervention. While pre-therapy tumours have a greater degree of proliferation compared to differentiation, the ratio is reversed after application of neoadjuvant chemotherapy, as evident from reduction in tumour size in response to therapy. The slow cycling of tumour cells post therapy compared to
Figure 6.1: Variation of the candidate genes during progression of head and neck tumours and therapeutic intervention

Variation of the normal basal/parabasal molecular signature of the candidate genes (expression/promoter methylation) in dysplastic epithelium and HNSCC and variation of the expression in shrunk tumours of the same patients after completion of neoadjuvant chemotherapy. While the basal signature of RBSP3 remains almost the same in tumours with slight increase in tumours post neoadjuvant chemotherapy, LIMD1 and CDC25A gradually shows alteration of the signature with gain of expression of LIMD1 post NACT.
untreated tumours was also confirmed by the decrease in proliferative index (PCNA expression) and cMYC, as well as by gain of expressions of tumour suppressors LIMD1 and RBSP3 and enhanced RB/ pRB ratio. Furthermore, decrease in tumour volume might be due to the evolution of a clone of cells more tolerant to the drugs used, as evident from the persistence of a small tumour mass after completion of chemotherapeutic cycle. This mass has a greater tendency to undergo apoptotic death, indicated by the increase in apoptotic index and BAX/ BCL2 ratio in post-therapy tumours compared to pre- therapy. Thus, all three cellular processes of proliferation, differentiation and apoptosis are perturbed when a tumour evolves from the basal proliferative layer and when it undergoes treatment due to neoadjuvant chemotherapy (Table 6.1).

Deletion of the candidate genes was an important event in the development of head and neck tumours. The CA repeat microsatellite markers associated with the genes and used for the study of their deletion are highly polymorphic and present several alleles, some of which might be more susceptibility to deletion (Section 4.4.1). The alleles present in the normal tissues adjacent to the tumour represent a wide range of CA repeats, in the range of (CA)9 to (CA)38 (Table 4.13). Interestingly, in tumours, alleles of the larger size were mostly deleted, probably due to greater instability of large sized alleles. Therefore, inherent and specific alleles present in the normal tissues, on exposure to etiological factors such as tobacco have a greater probability to undergo deletion, thus conferring susceptibility to the development of HNSCC. The presence of these alleles might in turn determine the response of a patient subjected to cancer therapy. While patients lacking the susceptible alleles might be able to tolerate a higher dose of drugs, patients harbouring such might undergo drug-related toxicity under the same scenario.

Thus, the role of the candidate genes RBSP3, LIMD1 and CDC25A in head and neck squamous cell carcinoma and their interplay is dependent not only on the alterations they undergo during tumorigenesis and chemotherapy, but also on the susceptible alleles they harbour. Screening of these parameters in individual patients prior to administration of treatment would help in planning therapeutic strategies tailor made for each individual patient, which would in turn provide more effective management of this deadly disease.
Table 6.1: Interplay of RBSP3, LIMD1 and CDC25A in HNSCC

Interplay of the candidate genes in the development of HNSCC and therapeutic response, as determined by expression of proliferation and apoptosis related proteins. During tumorigenesis, proliferation related proteins, including the candidate genes and related cell cycle proteins increased, while the expression of apoptosis related proteins remained low. In tumours subjected to neoadjuvant chemotherapy in the same patients, proliferation related proteins show reduced expression, while apoptosis inducer BAX showed higher expression, indicating a halting of the cell cycle and induction of apoptosis. Symbols (+/-) represent levels of expression: - : Very low or no expression; + : Low expression; ++ : Moderate expression; +++ : High expression.