8. FINAL DISCUSSION AND FUTURE DIRECTION

Although oesophagogastric cancer is traditionally associated with a poor prognosis, there is evidence that the outlook is improving. This is due to multiple factors that include: earlier diagnosis (due to open access endoscopy or Barrett's surveillance); better patient selection for appropriate treatment (surgery with either neo-adjuvant or adjuvant chemotherapy; or primary chemo-radiotherapy); improved intra-operative and post-operative management (such as high dependency and ITU support; improved nutritional support; early extubation and physiotherapy). However, to improve the prognosis of these cancers further it is necessary to understand the molecular mechanisms of carcinogenesis and disease progression. Hopefully, such research will highlight novel disease prevention and therapeutic strategies.

The central hypothesis underlying this thesis was that hypoxia plays a role in the aetiology and prognosis of oesophagogastric cancers.

8.1 Clinical prognostic factors in oesophageal cancer

8.1.1 Tumour length as a prognostic factor

In Chapter 3, I showed that tumour length is a valuable prognostic marker in oesophageal malignancy. Further confirmatory prospective studies of tumour length as a prognostic factor are required before it could be introduced as a routine histopathological prognostic factor. Encouraging preliminary results have been published determining tumour length preoperatively using either US or PET. Future research should be performed in a large cohort of patients measuring tumour length pre-operatively and correlating this with first the fresh surgical specimen and then the final histopathology analysis. It may be that this simple measurement can assist in the selection of patients for neo-adjuvant therapy. Towards this goal, our department is now investigating the role of CT estimation of tumour length and comparing this with other histopathological parameters and final patient outcome.

8.1.2 CRM involvement as a prognostic factor

Also in Chapter 3, I confirmed the results of other studies and showed that CRM is a valuable prognostic marker in oesophageal cancer. Currently there is enough evidence from this and other studies to continue to report CRM status on routine histopathology. However, the relationship between CRM and prognosis is not straightforward. I

showed that the prognostic outcome depends on the ratio of involved lymph node metastases.

It would be worthwhile evaluating CRM status in further prospective surgical and oncology studies. For example, research is required on the following aspects:

- Assessment of the prognostic outcome of tumour involvement from the CRM as calculated using a range of precise measurements.
- Comparison of whole mount histology sections assessing the CRM versus pre-operative staging imaging. For example, can CRM involvement be predicted by pre-operative imaging, such as CT, endoscopic US or MRI?
- Assessment of the impact of CRM on local recurrence in a large study.
- Assessment of the role of adjuvant therapy in patients with involved CRM.

8.2 Clinical prognostic factors in gastric and gastro-oesophageal junction cancer

In Chapter 4, a large database comprising all patients with GOJ and gastric adenocarcinomas who underwent surgical treatment in the SMUHT was developed and analysed. Prognostic factors were assessed using univariate and multivariate methods. This was an important prelude to using the database for molecular marker studies. The clinico-pathological and survival characteristics of GOJ versus gastric tumours were assessed. Our results confirmed the work of other studies showing that tumours differ in many aspects, including: clinico-pathology and prognosis. It is crucial for future research in this area that the classification of GOJ tumours is standardised and one system adopted internationally.

8.3 Molecular prognostic factors in gastric and gastro-oesophageal junction cancer

The prognostic impact of HIF-1α expression in gastric cancer appears to be dependent on the staining pattern; with HIF-1α expression at the invasive tumour edge associated with a poor prognosis. It was hypothesised that the difference in clinico-pathological and survival characteristics may be related to the differential regulation by HIF-1α of a range of downstream target molecules. Further work is planned to explore this hypothesis by performing immunohistochemistry to investigate apoptosis and proliferation in the same tumours. Although the work has failed to show independent

prognostic significance for the hypoxia associated markers HIF-1α and HIF-2α, there is still interest in incorporating them into multiple molecular studies. HIF-2α is potentially of interest as a target as high protein expression was observed.

In addition, future research is planned using resection specimens collected for prognostic marker staining. Rather than using whole mount specimens it is planned to use tissue micro-arrays (TMA) as this technology offers an efficient way of examining multiple proteins on a large number of tumour cases. One of the main benefits of the TMA method is greater uniformity of sample processing. Future work to be carried out in the department aims to assess a panel of molecular prognostic markers in TMAs. The tissue blocks used in this thesis have already been constructed as TMAs (Figure 8.1). Cluster analyses will be carried out to investigate potential marker profiles associated with a poor prognosis.

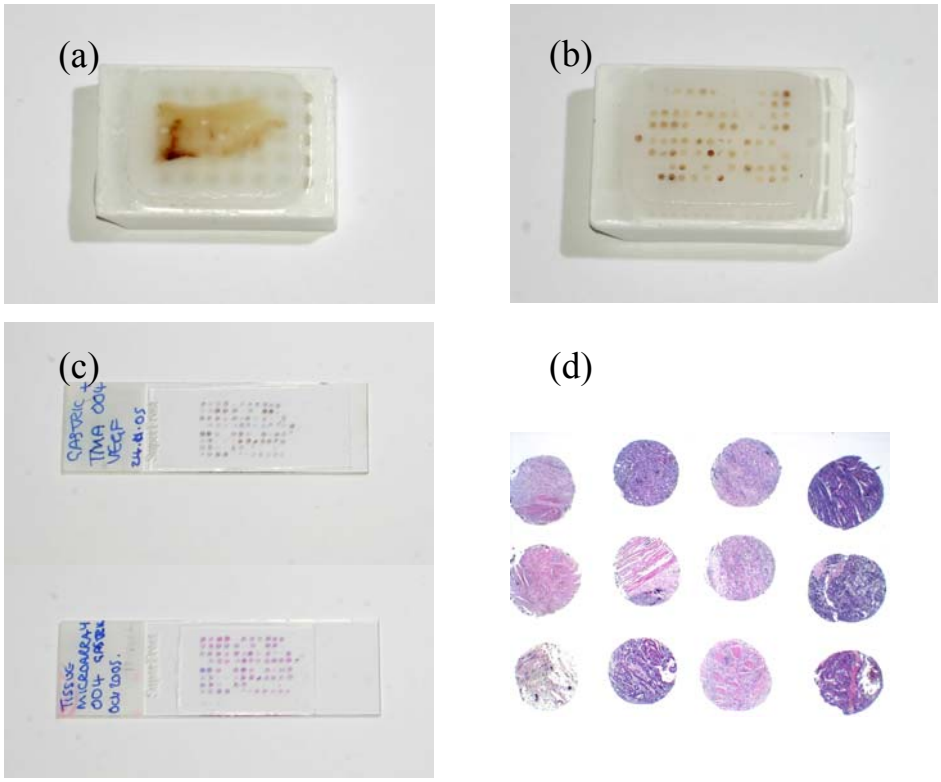

Figure 8.1 Construction of a TMA from specimen blocks used in this thesis.

(a) – Donor tumour block with several 1 mm cores removed from areas of interest (these were previously marked on a corresponding glass slide by a histopathologist); (b) – The TMA block is constructed by inserting each punch in order from the donor blocks; (c) – The H&E stained slides from the sectioned TMA; (d) – Close up view of the individual (H&E stained) TMA biopsies; (TMA were constructed using the MTA-01 Personal Tissue Arrayer [Mitogen, www.mitogen.co.uk, UK]).

8.4 Molecular markers in gastric and oesophageal carcinogenesis

HIF-2α was expressed only in patients with dysplasia or invasive adenocarcinoma of the oesophagus and not in earlier phases of the carcinogenesis sequence. It would be interesting to evaluate its ability to predict the risk of progression to malignancy in a cohort of patients with either low or high-grade dysplasia. This could be achieved by carrying out either a retrospective or prospective case-control study. Another future project could assess the discriminatory power and pathological usefulness of Glut-1 expression in helping to distinguish high-grade dysplasia from invasive malignancy in difficult cases.

Further studies to assess the possible role of hypoxia in carcinogenesis would be better achieved using cell line or other oesophagogastric cancer models rather than paraffin-embedded material. In this way, important environmental factors such as the levels of cellular hypoxia could be controlled for. For example, dose-response relationships could be calculated for a carcinogen, for example *H. pylori* concentration. This approach would allow the precise molecular biological pathways of carcinogenesis to be evaluated.

8.5 Prospective study assessing hypoxia in oesophagogastric adenocarcinoma

There is interest in microarray technology to study and develop mRNA expression profiles, which in the future could be used to influence therapeutic decisions in cancer patients (Sahar et al. 2005; Thompson 2005). The final aim of this prospective study is to develop and evaluate a 'hypoxia signature/profile' for oesophagogastric adenocarcinoma. This would be achieved by assessing the gene expression (from cDNA microarray technology) which is associated with hypoxia in these tumour biopsies. Similar work has been carried out by assessing hypoxic gene expression in several cell lines (renal tubular, breast epithelial, smooth muscle and endothelial cells) to develop a hypoxia signature (Chi et al. 2006). When this signature was applied to publicly available gene expression datasets it was a strong predictor of clinical outcome in both breast and ovarian cancer. The most interesting feature of this work was that in the breast cancer prognostic model the hypoxia signature was a strong independent predictor of clinical outcome. This was independent from the most useful clinico-pathological characteristics currently used for breast cancer, such as patient age, tumour size, lymph node metastases, grade, ER status and angioinvasion.

A similar approach was used in our department in a group of neck and neck cancer dataset (Figure 8.2). Affimetrix U113A GeneChips were used to profile RNA expression in 59 patients in a collaborative project involving the Weatherall Institute of Molecular Medicine, Oxford. A hypoxia associated gene signature cluster was obtained *in vivo* by clustering around the expression of 10 known hypoxia regulated genes. The median RNA expression of the 99 genes in the hypoxia signature was prognostic for recurrence-free survival in an independent head and neck derived cancer dataset, outperforming standard clinico-pathological variables and a trained intrinsic profile.

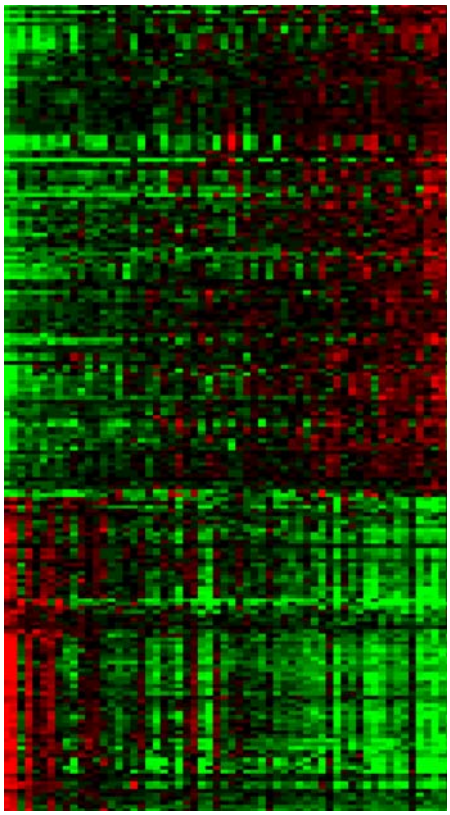

Figure 8.2 Dendogram from a study of 59 head and neck cancer patients.

Expression of up-regulated hypoxia associated genes (upper right hand quadrant) was a highly significant independent adverse prognostic factor in an independent dataset

This approach will be used in the samples collected in the prospective study to derive an oesophagogastric cancer hypoxia signature. The tumour specific hypoxia signature should prove useful for future studies aimed at increasing the biological understanding of, deriving prognostic markers for and suggesting new therapeutic targets in oesophagogastric cancers.