Molecular Detection of Occult Disseminated Disease in Breast Cancer Patients

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A thesis submitted in total fulfillment of the requirements
for the degree of Doctor of Philosophy

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July, 2003

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Abstract

A major clinical dilemma in the management of patients with early stage breast cancer is determining which patients are at risk of subsequent recurrence so that these patients may be offered adjuvant therapies. Currently, the major prognostic factors used in determining prognosis including nodal status and tumour size fail to identify up to 30% of patients classified as having low risk disease who subsequently recur with breast cancer. Therefore, there is a great need for new methodologies that can sensitively and specifically identify disseminated disease.

During this study, the major aims were to use immunobead-based and methylation-based methods for the detection of occult disseminated disease in peripheral blood samples obtained from breast cancer patients. Firstly, an RT-PCR assay was developed that identified a panel of gene specific markers that could be used for sensitive and specific detection of any circulating epithelial cells with minimal risk of false positive results from contaminating haematopoietic compartment cells. These gene markers were used to assess breast cancer patients using immunobead enrichment prior to RT-PCR (IB RT-PCR) and resulted in the detection of circulating epithelial cells in 21/57 peripheral blood samples. Importantly, some of these patients had been deemed lymph node negative and at low risk of relapse.

Furthermore, it was decided to screen for gene promoter region methylation in freely circulating DNA in the plasma fraction, and in DNA from the nuclei of epithelial cells captured using the immunomagnetic enrichment method. The analysis of gene loci methylation of patient samples was conducted concurrently with the analysis of the frequency of gene loci methylation in mononuclear cells from normal individuals. Surprisingly, a high proportion of normal individuals were methylated at high frequency at gene loci that have

previously been reported to be tumour specific. This finding had implications for the concurrent study using methylation as a marker of occult disseminated disease in breast cancer patients.

The analysis of blood samples using the methodology presented in this thesis for the early detection of breast cancer dissemination, has the potential to be developed to a point where it can be introduced into the clinical setting. Once fully developed and validated, this minimally invasive methodology, that causes little discomfort to the patient, could help to improve breast cancer staging, and provide important prognostic information ensuring the identification of those early stage breast cancer patients with the greatest risk of relapse and in most need of adjuvant therapies.