Candidate Tumour Markers and Potential Therapeutic Targets in Colorectal Cancer

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Abstract

Aim: To identify candidate tumour-specific molecular markers for the detection of disseminated tumour cells in peripheral blood and intra-peritoneal lavage samples from patients undergoing surgical resection for CRC and as potential therapeutic targets.

Results: cDNA microarray screening identified Dipeptidase-1 (*DPEP-1*) to be over-expressed by ≥ 2 fold in colon tumour compared to normal colonic mucosal tissue in 56/68 (82%) patients. The laminin gamma-2 chain of laminin-5 (*LAM-γ2*) and Matrilysin (*MAT*) were also identified as potential candidate molecular markers and found to be over-expressed in 22/30 (73.3%) and 47/53 (88.7%) patient matched samples respectively. Immunobead RT-PCR found *DPEP-1*, *LAM-γ2* and *MAT* positive cells in 82 of 168 (48.8%) CRC patients (14 Stage A, 32 Stage B, 17 Stage C and 19 Stage D). Of patients who were positive for one or more marker in any sample, 41 suffered disease relapse (recurrence) or death resulting from cancer progression within the follow-up period. Kaplan-Meier survival analysis, conducted on 110 early (A and B) stage patients, found those who were positive for any marker had significantly shorter disease-free survival than patients who were negative (P=0.026) and patients who were positive for any marker in their post-operative lavage samples also had a poorer survival outcome (P=0.038). Multivariate analysis showed that detection of disseminated tumour cells with any molecular marker remained significant (P=0.015, hazard ratio 3.459, 95% CI 1.272-9.410) and was independent of other risk factors of disease relapse, indicating patients that were positive for any marker were 3.5 times more likely to suffer relapse than patients who were negative. Further characterisation of *DPEP-1* and *LAM-γ2* identified that HT29 cells transfected with the *DPEP-1* construct migrated through a Matrigel™

invasion assay in greater numbers than untreated cells (P=0.007). RNA interference of *DPEP-1* found a significant difference in migration capacity between the mock transfected (MT) cells when compared to *DPEP-1* siRNA treated cells (P=0.034). Fluorescent immunohistochemistry located *DPEP-1* expression in the crypts of colon tumour tissue. Anti-LAM-γ2 treated LIM 2099 cells migrated through the Matrigel™ invasion assay in significantly reduced in numbers when compare to non-treated and normal IgG_1 antibody treated cells (P=0.0006) and siRNA-mediated gene silencing of *LAM-γ2* significantly reduced the number of cells migrating through the Matrigel™ invasion assay (P=0.007).

Conclusions: *DPEP-1* and *LAM-γ2* are potential targets for tumour-specific therapeutic intervention. Immunobead RT-PCR using a panel of molecular markers has the ability to identify early stage CRC patients at risk of disease relapse.