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Studies of cell migration and matrix protease production in human lung cancer cell lines

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Abstract

Metastatic spread in cancer is a complex multistep process involving continuous, sequential interactions between tumour cells and their respective microenvironment. Invasiveness *per se* has not been studied in this thesis, only some of its major components, such as cell migration and matrix protease production. To better understand some of these components, we used human lung cancer cell lines representing the four major histologic types: adenocarcinoma (Wart); squamous cell carcinoma (U-1752); small cell lung carcinoma (SCLC) (U-1906, 054A); and large cell carcinoma (U-1810).

Paper I concerns results of studies of cell migration using Boyden chamber assays. It was shown that cell lines Wart, U-1906, and 054A migrated chemotactically and haptotactically to components of the extracellular matrix - such as fibronectin, laminin and type IV collagen in a $\beta 1$ integrin-dependent fashion. In contrast, cell line U-1752 did not respond chemotactically to any of the three extracellular matrix components that were used. Fibronectin and type IV collagen induced chemotactic and haptotactic migration of the U-1810 cells, but laminin did not. Our study confirmed variable expression of integrins belonging to the $\beta 1$ family in human lung cancer cell lines, except in cell line U-1810 which did not express the $\beta 1$ integrin subunit. The migratory response differed depending on whether the chemoattractant was in a soluble or an insoluble form.

Paper II deals with studies of the expression of different growth-factor receptors and corresponding ligands in human lung cancer cell lines. Using RT-PCR, we found that IGF II/M6P, c-met, EGF and c-kit receptors are expressed in 5/5 cell lines. In order to investigate the biological function of these receptors, we performed Boyden-chamber assays using various growth factors as chemoattractants. The main result in this study was the finding that IGF I, IGF II, HGF, EGF and SCF stimulate migration of human non-small cell lung carcinoma (NSCLC) cell lines in a dose-dependent manner. In addition, checkerboard analysis demonstrated both a chemotactic and a chemokinetic response - indicating that these growth factors may act in both an autocrine and a paracrine fashion *in vivo*.

In studies reported on in paper III, we used gelatine zymography to demonstrate that three NSCLC cell lines express matrix metalloproteases (MMPs) -9 and -2. The expression and activity of MMP-9 and MMP-2 was heterogeneous in these cell lines. In addition, we showed that growth factors modulate MMP activity. HGF and EGF are capable of stimulating the conversion of MMP-9 from a latent to an active form in human large cell lung cancer cell line U-1810. Furthermore, IGF I, IGF II, HGF, and EGF stimulated an enhanced expression and activity of the latent form of MMP-2 and MMP-9. SCF did not enhance MMP activity in any of the cell lines that were tested.

In the studies described in paper IV, we found that cross-linking of $\alpha 2\beta 1$ on U-1752 cells with immobilized mAb induced motile behaviour in the absence of extracellular matrix components. In addition, it was shown that $\alpha 2\beta 1$ triggered migration was enhanced by growth factors - such as EGF and HGF. $\alpha 2\beta 1$ triggered migration could be blocked if the cells were pretreated with genistein, pertussis toxin or calphostin C, indicating the involvement of protein tyrosine kinases, G-proteins and protein kinase C-dependent signalling pathways, respectively.

Keywords: lung cancer, extracellular matrix, growth factors, matrix metalloproteases, integrins, migration, invasion

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