The secretory small GTPase Rab27B regulates invasive growth and metastasis in ER-positive breast cancer

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In contemporary oncological practice, the identification of patients with early stage breast cancer likely to benefit from adjuvant chemotherapy is challenging. This indicates an urgent need for prognostic refinement. Deciphering molecular pathways of how tumours invade and metastasise may help in the identification of a useful prognostic marker. Our research group recently discovered that the secretory small GTPase Rab27B, a regulator of vesicle exocytosis, delivers crucial signals for increased invasiveness, tumour size, and metastasis of various oestrogen receptor (ER)-positive breast cancer cell lines, both in cell culture and in xenograft mouse models. The presence of Rab27B protein proved to be associated with a low degree of differentiation and the presence of lymph node metastasis in ER-positive primary breast cancer. Rab27B is a potential key marker for stratification, prognosis and treatment of early stage ER-positive breast cancers which are more invasive and tend to metastasize.

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Introduction

Since the introduction of screening mammography a dramatic change has taken place in the presentation of breast cancer. The diagnosis of small ER-positive tumours without lymph node involvement has increased dramatically in screened populations throughout the Western world. Identifying patients with early breast cancer likely to benefit from adjuvant chemotherapy is difficult because time-dependent tumour characteristics (e.g. size and lymph node status) are generally less developed and less declarative of risk. Thus, in contemporary oncological practice, an urgent need remains for refinement of the prognostic assessment of breast cancer.

Communication in the tumour environment drives metastasis

Although local infiltrative tumour growth and distant metastasis are the main prognostic denominators in tumour malignancy, our diagnosis is often inadequate to cope with these activities. Both depend upon the delivery of pro-invasive growth regulators into the local and distant ecosystem.

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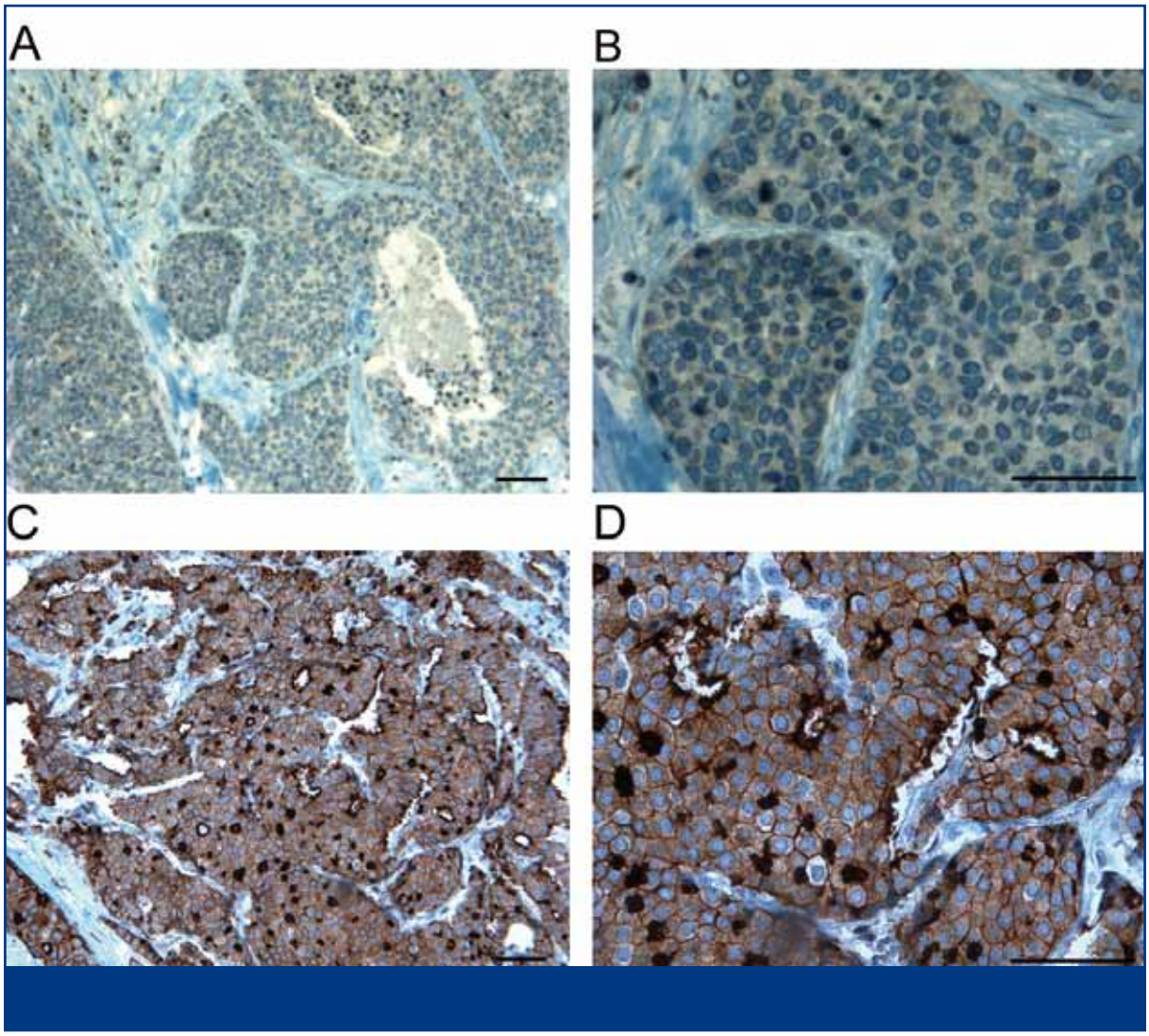

Figure 1. Rab27B protein expression in primary breast cancer samples. A and B. Estrogen receptor (ER)-negative primary breast cancer. C and D. Poor prognosis ER-positive primary breast cancer with an accumulation of the Rab27B signal at the cell periphery, suggesting a secretory function. Scale bar: 100 nm.

Cancer is not only a disease of the transformed epithelium but is also influenced by and dependent on its stromal environment. So, secreted pro-invasive growth regulators from both the cancer cells and the stromal cells may determine invasion and metastasis. Work from our group demonstrated a crucial role for tumour-associated myofibroblasts in invasive growth.[1,2] Tumour-associated myofibroblasts and their bone marrow-derived precursors are attracted by cancer cells and accumulate at the cancer invasion front.[3,4] However, much less insight has been gained into the regulatory networks establishing secretion of pro-invasive growth regulators.[5] One likely process involves vesicle exocytosis, controlled by secretory GTPases such as Rab27B, Rab27A and Rab3D. Rab GTPase mediated vesicular transport is an attractive candidate for upstream regulator of metastatic progression because secretory vesicles can contain entire sets of pro-invasive factors, including messenger (m) RNAs and micro (mi)RNAs.

Presence of Rab27B in primary human breast tumours

A quantitative real-time PCR screen of secretory Rab GTPases on 20 primary breast cancer samples revealed a 10-fold increase in Rab27B messenger

(m)RNA expression in tumour tissue compared to normal tissue. Rab27B mRNA levels were significantly higher in ER-positive primary breast cancer with lymph node metastasis. Rab3D and Rab27A mRNA levels did not differ significantly. An immunohistochemical analysis of 59 primary breast cancer specimens, using a specific polyclonal Rab27B antibody, demonstrated a significant correlation among high Rab27B expression, the presence of lymph node metastasis, and poor differentiation in ER-positive breast cancer. In these tumours, Rab27B localised at the cell periphery, suggesting a secretory function. ER-negative breast cancers showed no signal for Rab27B (*Figure 1*). Microdissection of epithelial cancer nests from fresh-frozen primary breast cancer tissue was used to quantify the Rab27B protein levels. These observations were indicative to engineer ER-positive breast cancer cells (such as MCF-7, T47D) that overexpress green fluorescent protein (GFP) fused with Rab27B to similar expression levels and localisation patterns found in invasive ER-positive breast tumours. [5]

Rab27B stimulates matrix invasion and proliferation in cell culture models

Breast cancer cells in which Rab27B was overexpressed formed cellular extensions and a spread morphology. These morphological changes resulted in an increased ability to invade Matrigel and native type I collagen substrates, representing the basal membrane and the extracellular matrix respectively. In addition, Rab27B enhanced proliferation under limiting serum concentrations, through increased G1- to S-phase cell cycle transition.[5]

Rab27B promotes invasive growth in mouse models

In xenograft mouse models, Rab27B promoted invasive growth. This finding was evidenced by increased tumour volume, tumour weight, and Ki67 proliferation index, and by the massive infiltration of cancer cells into the abdominal skeletal muscles. Shedding of cancer cells from the primary tumour into the peritoneal cavity occurred. Anchorage-independent metastatic cells were present both as single cells and multicellular aggregates in the peritoneal cavity. Finally, 15 out of 40 mice bearing Rab27B xenografts succumbed to haemorrhagic ascites formation.[5]

Molecular mechanisms

How does Rab27B achieve invasive growth? To search for pro-invasive growth regulators released upon Rab27B-mediated secretion, we established a novel method for intracellular Rab-associated whole-vesicle isolation. Proteomic analysis on purified intracellular Rab27B vesicles identified the pro-invasive growth regulator heat shock protein (HSP)90 alpha. Extracellular HSP90 alpha plays a crucial role in the activation of matrix metalloproteases, enzymes for extracellular matrix degradation. Analysis of the conditioned medium of the breast cancer cells, demonstrated a Rab27B-dependent release of HSP90 alpha. Neutralising HSP90 alpha antibodies abolished Rab27B-induced collagen type I invasion and proliferation. In a supportive experiment, recombinant HSP90 alpha protein stimulated both functional responses. Gelatin zymography, a technique used to measure proteolytic activity, showed a Rab27B and HSP90 alpha-dependent activation of MMP-2.[5]

Conclusion

Rab GTPase mediated vesicle exocytosis is efficiently implemented by cancer cells to relay crucial information in the tumour ecosystem for fostering growth, migration and matrix degradation.[6] Increased expression of Rab27B is associated with poor prognosis in ER-positive breast cancer. Rab27B transports vesicles containing the pro-invasive growth regulator HSP90 alpha to the cell membrane. After membrane fusion HSP90 alpha is released in the tumour environment leading to the activation of MMP-2 protease. This results in degradation of the extracellular matrix and growth factor release, and as a consequence invasive growth.[5,6]

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Key messages for clinical practice

1. Breast cancer is a heterogeneous disease and its biological complexity is a major challenge for translational research. Molecular profiling revealed at least 2 distinct subtypes in the ER-positive breast cancer group.

2. Additional well-defined biological markers could improve breast cancer sub-classification and the accuracy of prognostic and therapeutic decisions for a tailor-made therapy. The use of markers with an affordable and widely spread technology, such as immunohistochemistry, is useful. Rab27B is a potential key marker for stratification and prognosis of early stage ER-positive breast cancers which are more invasive and tend to metastasise more frequently.

3. Considerable therapeutic potential may reside in efforts to control Rab27B levels and to modulate Rab27B-regulated pathways through pharmacological or genetic interventions.

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