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Original contribution

A favorable role of prolactin in human breast cancer reveals novel pathway-based gene signatures indicative of tumor differentiation and favorable patient outcome☆,☆☆

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Summary Prolactin (PRL) hormone is known to play a key role in mammary gland development allowing for successful lactation. The role of this hormone in breast tumorigenesis is still controversial. Here, we evaluated PRL protein and gene expression levels in human breast cancer using tissue microarray of 100 breast cancer cases, as well as different publically available human breast cancer gene profiling databases. Interestingly, our results showed a significant downregulation of PRL expression in breast cancer compared to normal adjacent tissue. Moreover, expression of PRL was associated with more differentiated tumors, early stage, smaller tumor size and absence of distant metastasis. Importantly, our results indicate that higher PRL mRNA levels are significantly associated with prolonged relapse-free survival (RFS) in breast cancer patients ($P = 3.7 \times 10^{-9}$). Additionally, examining expression of PRL pathway-based gene signature composed of PRL, PRLR, Jak2 and Stat5a showed a significant association with more differentiated tumors ($P < .00001$), prolonged RFS ($P = 1.8 \times 10^{-6}$) as well as overall survival (OS) ($P = .0026$). As well, our results indicate that PRL-directed differentiation program in mammary epithelial cells offer good prognosis in human breast cancer. Indeed, expression of a gene signature composed of PRL-upregulated genes showed a significant association with well-differentiated tumors ($P < .00001$). Whereas expression of a gene signature composed of PRL-downregulated genes showed a significant association with shortened distant metastasis-free survival (DMFS) ($P = .0086$). Altogether our results highlight that PRL hormone and its signaling pathway may play an important role in maintaining tumor differentiation state and in turn better patient outcome.

Abbreviations: DMFS, distant metastasis-free survival; FBS, fetal bovine serum; HIPAA, Health Insurance Portability and Accountability Act; IRB, institutional review board; OS, overall survival; PRL, prolactin; PRLR, prolactin receptor; RFS, relapse-free survival; TMA, tissue microarray.

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1. Introduction

Mounting evidence suggests that the process of cellular differentiation confers the cells with properties making them resistant to transformation and hence tumorigenesis. Clinically, it is observed that higher tumor grade is usually associated with lower long-term patient survival [1,2]. Indeed, the degree of tumor cell differentiation is an important parameter for prognosis in breast cancer patients. These observations implicate cellular differentiation pathways as pivotal in regulating the biological behavior of tumor cells and thereby patient outcome. In spite of this important role, most of tumor biology studies have focused on molecular pathways involved in cellular growth, survival, migration and invasion. So far few studies have focused on cellular differentiation processes and their association with tumorigenesis [3].

In breast tissue, PRL hormone through activation of the PRLR/Jak2/Stat5 pathway is known to play a key role in mammary gland development and terminal differentiation of the mammary epithelial cells during lactation [4,5]. Previous studies suggested that PRL plays a role in the growth of breast cancer cells through PRL/PRLR autocrine function [6,7] leading to promoting breast cancer tumorigenesis. However recent study has shown that PRL expression in breast cancer cell lines and tissues to be low/undetectable [8]. We have previously shown that PRL via its main downstream tyrosine kinase Jak2 regulates epithelial plasticity of breast cancer cells leading to suppression of their mesenchymal and invasive phenotype [9]. Furthermore, our recent study showed PRLR expression in human breast cancer to be associated with well-established good prognostic clinicopathological parameters suggesting that PRL may play a tumor suppressor role in breast cancer [10]. In accordance with these findings expression/activation of the PRL effector molecule Stat5a was shown to associate positively with increased levels of histologic differentiation of breast cancer tissues and distinguishes breast cancer patients with favorable prognosis and response to endocrine therapy [11,12].

Here, we aimed to evaluate the local expression of PRL in human breast cancer cases in comparison to normal tissue. Moreover, we investigated the association between PRL expression levels and well-established clinicopathological parameters. Finally, we evaluated the association between the PRL signaling pathway as well as PRL-modulated target genes in relation to patient outcome. Together these studies should help clarify the role of PRL in breast cancer.

2. Materials and methods

2.1. Cell culture

HC11 mouse mammary epithelial cells (obtained from N. Hynes, Friedrich Miescher Institute, Basel, Switzerland, 1996) were cultured in RPMI 1640 supplemented with 10% FBS, mEGF 10 ng/ml, and insulin 5 μg/ml. The human breast cancer cell line T47D (obtained from Dr. Morag Park) was cultured in DMEM supplemented with 10% FBS.

2.2. Immunohistochemistry

TMAs of 110 cores were obtained from US BIOMAX Inc. (100 cases of invasive ductal carcinoma with various grades and stages plus 10 cases of normal adjacent tissue. BIOMAX assured that all patients gave a written informed consent and all the tissues were collected with high ethical standards under the HIPAA-approved protocols. For that reason IRB approval was not necessary. All the cases were reviewed by an anatomic pathologist to ensure that the cores are pathologically representative of the original tumors and the clinicopathological information was accurate. In brief, slides were deparaffinized and then rehydrated. After antigen retrieval, staining was performed using Ultravision LP Detection System (Lab Vision Thermo, USA). Antibodies used in this study: PRL (Santa Cruz #sc-7805), PRLR-L (Santa Cruz #sc-20,992). Positive controls included the human breast cancer cells lines T47D and MCF7. All TMA slides were scanned using a digital image capture system, the Aperio XT slide scanner (Leica Biosystems) at the core facility, Breast Cancer Functional Genomics Group, Goodman Cancer Research Centre, McGill University. Two investigators evaluated immunostaining independently and in a blind manner from the clinicopathological data. When there was a difference in evaluation, simultaneous examination by both investigators was done to resolve the discrepancies.

For PRL and PRLR analyses, we used a semiquantitative scoring system as previously described [13,14]. A score of 0 indicates undetectable expression. Expression in $<10\%$ of tumor cells, was considered as (+1). Expression in 10%–50% of tumor cells was considered as (+2), and if expression was detected in $>50\%$ of cells it was considered as (+3). Both (0) and (+1) scores were considered negative. The staining was considered positive only if there was membranous and/or granular cytoplasmic staining in malignant cells.

2.3. Kaplan-Meier survival analysis

Breast Cancer Kaplan–Meier plotter (2014), which includes 4142 breast cancer patients, was used to plot patient outcome represented as RFS and DMFS and OS [15]. Cohorts of patients were separated by median expression using auto-selected best cut-off. The PRL pathway gene signature was obtained using the mean expression levels of PRL, PRLR, Jak2 and Stat5a using the multigene classifier tool of KM plotter, while the PRL-modulated gene signatures was obtained using the mean expression levels of PRL-upregulated genes and PRL-downregulated genes using the same multigene classifier tool in the KM plotter.