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New Doctorial Cancer Research

Molecular Analysis of Progesterone Receptor B (PRB) in Breast Cancer

Orla Mc Cormack

UCD School of Medicine and Medical Science,
UCD Conway Institute, Belfield, Dublin 4, Ireland

Orla Mc Cormack

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Supervisor: Professor Michael Kerin

Steroid receptor studies in breast cancer are clinically significant because they determine hormonal treatment modalities. However, despite extensive work on estrogen receptor alpha, there is continuing clinical debate as to whether these markers alone can help identify those patients more likely to respond to tamoxifen. Progesterone receptor status has contributed useful information in defining more responsive subgroups. Progesterone acts through two progesterone receptors (PRs), PRA and PRB. PRB is the functionally active PR, and can be silenced by promoter hypermethylation. ER alpha acts as a transcriptional activator of PRB. Thus, PRB status in tumors is important in assessing hormone receptor pathway activity. PRB upregulates a number of distinct genes involved in breast cancer mitogenesis, including growth arrest–specific gene 6 (Gas6), which encodes for the ligand of the Axl tyrosine kinase receptor. Overexpression of Gas6 results in the stabilization of beta-catenin (an E-cadherin anchoring protein) in breast cancer, implying a possible role in the development of features of the epithelial mesenchymal transition (EMT) phenotype, a well-recognized phenomenon resulting in increased tumor invasiveness, proliferation, and cancer cell motility.

Experimentally, following DNA and RNA extraction from 94 breast carcinomas, the methylation status of the PRB promoter was assessed by sodium bisulphite modification, and methylation-sensitive PCR (MSP), using previously published primers. A quantitative real-time PCR analysis (QRTPCR) was optimized and used to accurately determine the levels of PRB and Gas6 mRNA expression in the tumor cohort. Protein expression was evaluated immunohistochemically with a commercially available PRB-specific monoclonal antibody.

From the MSP results, there was a high degree of promoter methylation in the cohort, with 76% (71/94) of the primary breast carcinoma samples showing a methylated band for PRB. However, there was no significant correlation between PRB methylation profiles and PRB mRNA expression levels, or between PRB methylation profiles and PRB immunohistochemistry (IHC). PRB mRNA levels correlated positively with PRB IHC ($p = 0.002$). PRB methylation did significantly compromise total PR IHC expression ($p = 0.03$), and was associated inversely with tumor size ($p = 0.001$), and the amount of DCIS in the tumor ($p =$

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0.02). PRB mRNA correlated positively with total PR IHC ($p = 0.04$, $r = 0.58$), ER alpha IHC ($p < 0.001$), tumor size ($p = 0.003$), and tumor grade ($p = 0.01$). PRB protein expression was significantly associated with a number of good prognostic clinical variables including small ($p = 0.004$), low-grade ($p = 0.007$), ER alpha IHC–positive tumors ($p < 0.001$), and tumors that had a low NPI score ($p = 0.003$).

PRB methylation status and PRB IHC levels were not significantly associated with the disease-free interval or overall survival on univariate analysis. However, PRB mRNA levels were significantly associated with better overall survival ($p = 0.04$). In multivariate analysis, stage was the only predictor of disease outcome.[1]

Gas 6 results were analyzed with our previously obtained PRB profiles. There was a positive correlation between PRB IHC and Gas6 ($p = 0.036$). Gas6 mirrored the role of PRB in the cohort, and correlated inversely with poor prognostic markers, indicating that high levels of Gas6 corresponded with smaller ($p = 0.02$), well-differentiated tumors ($p = 0.03$). Gas6 was not significantly associated with the disease-free interval or survival on univariate analysis.[2]

In conclusion, a substantial proportion of tumors was methylated for PRB. When PRB was expressed, it correlated with good prognostic markers and better overall survival. There was a significant association between PRB and Gas6, and when Gas6 was expressed, correlations with good prognostic outcome were similar to those found for PRB.

REFERENCES

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Comment by Dr. Amanda Mc Cann, Ph.D.

UCD School of Medicine and Medical Science, UCD Conway Institute of Biomolecular and Biomedical Research, UCD, Belfield, Dublin 4, Ireland

This MD thesis focuses on progesterone receptor B (PRB) using a detailed molecular approach to investigate the mechanisms and activation of the gene-coding

PRB. This is the first study to specifically look at the promoter hypermethylation patterns of PRB in human breast cancer using sodium bisulphite modification and subsequent MSP, relating the findings to PRB real-time quantitative mRNA analysis, PRB immunohistochemistry clinicopathological characteristics, and patient outcome.

Overall, the study has shown that increased PRB levels are associated with favorable prognostic parameters and PRB negativity with adverse tumor features, the latter as a result of promoter hypermethylation or other as yet undefined mechanisms.

In relation to the Gas6 data, Gas6 expression correlated significantly with PRB expression in this cohort of patients, representing the first documented positive relationship between PRB and Gas6 in human breast tumors extending the previous breast cancer cell line work by Richer et al. (Richer JK, et al., 2002 PMID: 11717311).

Gas6 is an attractive target for molecular manipulation because it is the ligand for the Axl tyrosine kinase receptor, which has previously been shown to be overexpressed in a subset of breast cancers and other hormone-responsive tumors. Further work examining the role of an agent, which targets Gas6 or Axl, would benefit women with breast cancer by blocking a downstream effector of progesterone and potentially decreasing thrombosis without causing increased bleeding. Theoretically, such a strategy could be used with conventional anti-estrogen agents such as tamoxifen and aromatase inhibitors.