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Immunoglobulin kappa chain as an immunologic biomarker of prognosis and chemotherapy response in solid tumors

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Infiltration of plasma cells is associated with better prognosis in breast, lung and colon cancer. Immunoglobulin κ chain (IGKC) is now available as a single, robust immune marker predicting metastasis-free survival and response to chemotherapy. This will facilitate a deeper understanding of the role of the humoral immune system in cancer development.

Keywords: breast cancer prognosis, immunoglobulin kappa C, humoral immune system, prognosis, prediction of chemotherapy response, lung cancer, colon cancer

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Breast Cancer Prognosis and the Relevance Biomarkers

Defining risk categories among cancer patients is of considerable clinical relevance and will avoid over, as well as undertreatment. The most important factor for risk stratification in primary breast cancer is nodal status. Age, tumor size, estrogen receptor (ER) status and histological grade are all useful factors to further improve the outcome prediction. These "traditional" prognostic factors have been included into several outcome classification systems, e.g., the NNBC-3 risk algorithm.[1] The advent of genome-wide gene expression analysis has undeniably improved the possibility to identify breast carcinomas that progress to metastasis.[2-5] Some of the established gene expression signatures have been included as part of the clinical routine. Most of these predictive classification algorithms predominantly rely on ERα-regulated genes and/or on genes involved in proliferation.[6-11]

The Discovery of Biological Motifs in Gene Expression Patterns

Gene expression profiling also helped researchers recognize the heterogeneous nature of breast cancer.[12-14] As a result, breast cancer is now classified into the five molecular subtypes: luminal A, luminal B, basal-like, normal-like and HER2-like.[12,15] A subsequent milestone was the discovery of biological motifs.[16] Unsupervised clustering of gene expression data of breast carcinomas demonstrated the existence of highly correlative sets of genes representing either specific biological processes or specific cell types. The already well known influence of proliferation and ER receptor status was confirmed by the presence of two gene clusters consisting of genes involved in cell cycle progression and genes controlled by ERα.[16] Interestingly, the importance of a further, so far unrecognized, gene cluster consisting of immunoglobulins and other B cell/plasma cell-associated genes was discovered. A normalized mean of this 60-gene B-cell/plasma cell cluster was introduced and named the "B-cell metagene."[16-17] High expression of the B-cell metagene is associated with better prognosis in breast cancer, particularly in highly proliferating carcinomas.

Immunoglobulin κ Chain Surfaces to the Top

A limitation of the B-cell metagene is that the analysis of 60 genes is excessively laborious for routine clinical practice. Moreover, fresh frozen tissue for RNA isolation is often not available. Therefore, biostatistical analyses were performed to identify the top single biomarkers among the 60 gene of the B-cell metagene, considering prognostic power and the dynamic range (the range between low and high expressing carcinomas). Immunoglobulin

κ chain (IGKC) was identified as one the top genes,[18] and when validated in further cohorts, was found to be as predictive as the entire B-cell metagene. The availability of IGKC antibodies that could be used to immunostain paraffin slices also supported the use of IGKC use as a biomarker. With these immunostainings, carcinomas with strong IGKC immunostaining (3+, 2+) can easily be differentiated from negative (0) tissues (**Fig. 1A**). IGKC immunoreactivity, particularly IGKC 3+/2+ vs. 0, predicts prognosis that is consistent with the predictions based on RNA expression (**Fig. 1B**). Co-staining with the plasma cell/plasma blast marker MUM1 demonstrates that tumor-infiltrating plasma cells or plasma blasts are the source of IGKC expression (**Fig. 1C**). In contrast, $CD20^+$ B cells, T cells and tumor cells are $IGKC^-$.[18] It should be considered that it is the presence of infiltrating plasma cells that is relevant for the association with prognosis, and not the expression of IGKC itself, since other immunoglobulins, e.g., immunoglobulin λ, are also associated with prognosis.[16] However, IGKC is particularly convenient as a biomarker for statistical reasons, as reflected by its high dynamic range, and practical reasons, due to the availability of a reliable antibody. We also tested antibodies directed against other plasma cell constituents (e.g., CD138) and obtained significant associations with prognosis. However, the evaluation is more difficult, because the anti-CD138 antibody also stains tumor cells. In contrast, the anti-IGKC antibody exclusively labels infiltrating plasma cells/plasma blasts, leading to unequivocal and easy-to-interpret results (**Fig. 1**).

After analyzing data from over 1,800 breast cancers, 1,000 non-small cell lung cancers and 500 colorectal carcinomas, it is now quite evident that the prognostic relevance of IGKC is not limited to breast cancer. It represents indeed a universal, single, robust immune marker for clinical-scale testing.[19]

IGKC Predicts Response to Neoadjuvant Chemotherapy in Breast Cancer

Biomarkers that help to decide whether patients benefit from chemotherapy are

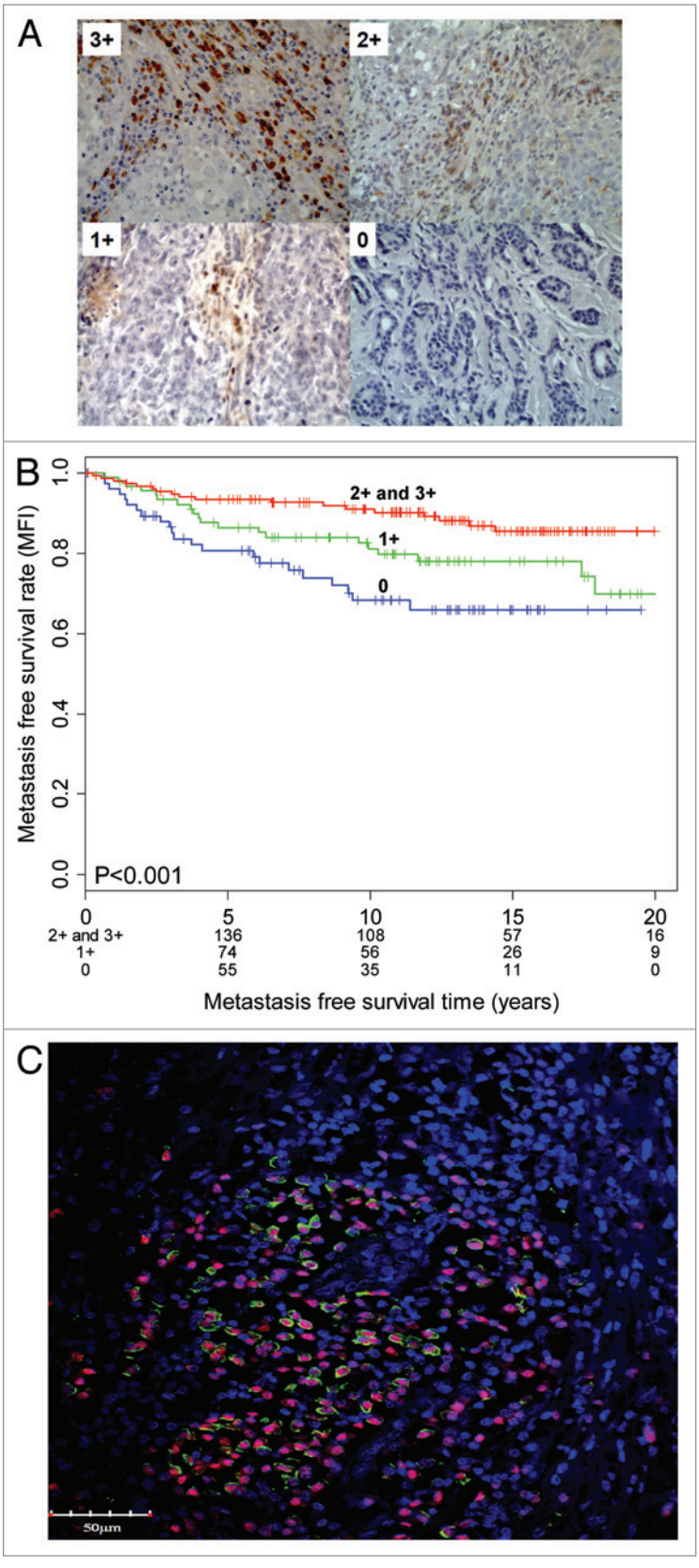

Figure 1. (**A and B**) Immunoglobulin κ chain (IGKC) expression (**A**) is associated with metastasis-free survival in breast cancer (**B**). (**C**) IGKC (green) is expressed in tumor-infiltrating plasma cells. The plasma blast/plasma cell marker MUM1 is visualized by red nuclear staining (adapted from ref. 18).

urgently needed in oncology.[19] Today, only a few such markers are available, and none fits into the immune marker category.[19] Importantly, IGKC does not only predict metastasis, but is also associated with response to chemotherapy, as demonstrated by the analysis of 845 breast cancer patients receiving anthracycline-based chemotherapy.[18] Again, a comparison of IGKC as a single marker compared with the entire B-cell metagene yielded similar results. A possible explanation for this observation is that tumor cell killing by chemotherapy releases antigens that trigger immune responses. In conclusion, the introduction of IGKC into the clinical practice may fulfill the urgent need to identify patients who will profit from chemotherapy.

Future Perspectives: Understanding the Janus-Faced Immune System

Considering the complexity of mechanisms controlling metastasis and chemosensitivity, the hunt for useful biomarkers has just begun. Biomarkers of other possibly relevant mechanisms, such as redox control,[20] mechanoactivity[21] and the tumor metabolome and lipidome[17,22,23] still need to be evaluated. However, a perspective for the near future is that we are close to fully understanding the mechanisms responsible for the Janus-faced nature of the immune system. With respect to prognosis of tumor patients, a favorable but also detrimental influence of the humoral immune system has been described (reviewed in ref. 10). The favorable influence has recently been harnessed by therapy with tumor antigen-specific antibodies, such as trastuzumab, rituximab or cetuximab.[19] Similarly, antibodies produced by tumor-infiltrating plasma cells may bind to tumor antigens and mediate antibody-dependent cellular cytotoxicity.[19] On the other hand, experimental studies and clinical data indicate that humoral immune responses may also mediate pro-tumor effects.[24,25] This is likely due to cytokines released from immune cells that stimulate the proliferation of tumor cells. As described in this article, both the entire B-cell metagene (defined as a normalized mean of 60 genes) and its single representative IGKC are associated with improved prognosis. Interestingly, a minority of genes within the B-cell metagene are clearly associated with worse (!) survival. Do they indicate a subtype of B cells that have turned "evil" and now initiate pro-tumor effects? Differentiating good and evil components of the humoral immune system in the future will further help in the selection of treatments that are most beneficial to cancer patients.

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