

A Special Key for Unlocking the Door to Targeted Therapies of Breast Cancer

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The best hope for cancer therapy is currently thought to be personalized medicine and targeted therapies. Proof of principle has been shown with the drugs Gleevec® (bcr-abl) for chronic myeloid leukemia (CML) and Herceptin® (Her2-neu) for breast cancer. Both of these target a receptor that sits on the outside of cancer cells, much like the lock that opens a door. Receptor tyrosine kinases have come to fruition as therapeutic targets in a variety of malignancies. In particular, c-Met (<http://www.vai.org/met/>) and its ligand HGF/SF have gained considerable interest because of their role in growth, morphogenesis, and motility, as they work together to transform normal cells into malignant cells. The processes of cell motility, invasion, metastasis, epithelial to mesenchymal transition, angiogenesis, and tissue regeneration all involve c-Met and HGF/SF[1]. In breast cancer, their deregulation promotes cancer progression and prognosis[2,3,4,5,6].

Clinicians have become increasingly better at recognizing patients at risk for breast cancer (BRCA1, etc.), wide-spread screening programs improve early diagnosis, and instrumentation enables clinicians to sample these early and small lesions. Thus, what used to be a disease of invasive cancer is now a disease of an earlier and more difficult to define biologically *in situ* stage. Patients and clinicians need to know what is an objective and measurable feature in order to define the biological potential of these early and precursor lesions, and what will be a therapy particular to their lesion.

Ductal carcinoma *in situ* (DCIS) of the breast is the immediate precursor lesion of invasive breast cancer and therapeutic strategies have been more aggressive due to high recurrence rates. There is a strong need to direct therapy in a more precise matter and here c-Met is an intriguing target. Deregulated activation of the c-Met pathway in transformed cells has led to the development of multiple approaches for targeted therapies, and a variety of c-Met pathway inhibitors have been identified that not only target c-Met itself, but also disrupt the interaction of c-Met with its natural ligand HGF/SF. In particular, those breast cancers that are hormone receptor negative and Her2-neu negative (so called triple negatives) are becoming more prevalent, and there is no effective targeted therapy for them short of invasive surgery

and nonspecific radiation as well as toxic chemotherapy. It is predicted that molecularly targeted therapy against c-Met will lead to dramatic inhibition of cancer growth and metastasis.

We recently published a study in *Histopathology* on the role of both c-Met, its ligand HGF/SF, and Her2-neu in DCIS of the breast[7]. Analysis of c-Met expression identified highly aggressive lesions, independent of Her2-neu expression. Both, up- and down-regulation of c-Met expression compared to surrounding normal tissue characterized a phenotype with unfavorable histopathology, such as Van Nuys (VN) Grade 3. DCIS cases classified as VN3 are considered to be high-grade lesions and are associated with a greater risk of recurrence[8]. We therefore believe that c-Met may be the key to understand DCIS aggressiveness and malignant progression, as well as a marker for targeted therapy in *in situ* carcinoma.

In breast cancer, c-Met overexpression is associated with tumor progression and poor survival. Its prognostic value has been demonstrated in early-stage patients with negative lymph nodes[9,10]. There are only a few studies on c-Met expression in DCIS, but they may elucidate the role of c-Met-HGF/SF signaling in early tumor progression. Jin et al.[11] observed significant stronger staining for c-Met and HGF/SF in DCIS than in benign hyperplasia, yet lower levels than in infiltrating carcinomas. Götte et al.[12] found c-Met overexpression more frequently in a subgroup of pure DCIS than when associated with a coexistent invasive carcinoma. This may contradict another finding of the study that c-Met exhibits a positive correlation with features of aggressiveness, such as HER2-neu overexpression, and may rather be indicative of the increased angiogenic activity in pure DCIS. Progressive loss of E-cadherin expression and a progressive increase in c-Met expression were observed with increasing dedifferentiation and higher metastatic potential. The prognostic impact of Her2-neu overexpression in invasive breast cancer has been widely demonstrated[13,14,15]. Its overexpression is even more frequently observed in DCIS than in invasive tumors[16,17], but its role in the progression to invasive disease is not completely understood. As Her2-neu overexpression may not play a key role in the progression of DCIS to invasive carcinoma[18], the investigation of c-Met expression may contribute to the understanding of their role in tumor development and progression.

In our study, the expression of both c-Met and Her2-neu in DCIS of the breast was analyzed for the first time using two independent immunocytochemical staining techniques. Most results obtained by conventional immunohistochemistry were confirmed by confocal immunofluorescent analysis. We did not observe a significant correlation between c-Met and Her2-neu overexpression, indicating that c-Met expression in DCIS is independent of Her2-neu overexpression. Analysis of c-Met expression identified highly aggressive lesions with an imbalance in c-Met expression between tumor lesion and surrounding tissue. This is consistent with the hypothesis that tumors with an imbalance between c-Met expression in tumor vs. normal tissue are less well differentiated and have a higher proliferative activity[19]. Recently, Shattuck et al.[20] even showed how breast cancer cells use c-Met receptor signaling as a method to overcome trastuzumab resistance. There were no obvious signs of intense tumor-stroma interaction with regard to HGF/SF expression in our study. The fact that HGF/SF is the essential ligand for metastasis and that paracrine secretion might contribute to an invasive behavior leads to the assumption that this missing secretion contributes to the inability of the lesions to degrade the basement membranes. Our results suggest that the balance between c-Met expression in the tumor and adjacent normal tissue, together with a paracrine HGF/SF secretion, could be the key to understand the invasive potential in DCIS.

The overexpression, mutation, and amplification of c-Met in tumor cells underline its pleiotropic functions and hint at c-Met as an ideal target in clinical therapeutics. This has led to the development of multiple approaches for targeted therapies both by inhibition of c-Met or c-Met peptides[21,22,23,24], inhibition of HGF/SF activation of c-Met[25], or targeting c-Met expression at RNA level[26,27]. The reported study suggests biologic features, such as c-Met expression, which may help to identify highly aggressive DCIS lesions, which require larger excision margins, more extensive local and medical treatment, or a different follow-up.

Further studies on the role of c-Met and HGF/SF in the transition from DCIS to invasive carcinoma are needed in order to understand which factors will influence this balance, and how c-Met and HGF/SF are regulated during tumor progression. Efficient therapeutic approaches may need to target simultaneously multiple molecules that are relevant to this early step of breast cancer progression. In the

future, we hope to be able to determine if c-Met-HGF/SF inhibition is an effective cancer treatment for DCIS as it is expected to be an important signaling target in a large number of malignancies.

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