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Aberrations of growth factors as biomarkers of cancer progression

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ABSTRACT

Cell proliferation and growth are regulated by a complex network of growth factor and growth inhibitor-initiated signal transduction pathways. The disruption of these signaling pathways through genetic, epigenetic, or somatic alterations is a major area of cancer research. Increasing evidence indicates that oncogenic activation of growth factors and their receptor proteins occur through mutations (oncoproteins) that lead to constitutive activation of the signaling pathways, thus providing the grounds for putative prognostic marker(s) and potential target(s) for treatment of various cancers. Over the past few years, the study of genomics has revealed the gene expression signatures for many malignancies. Present communication outlines literature survey on genomic molecular markers of breast, lung, and prostate cancers. Reassuringly, the dominant genomic markers of these malignancies include oncoproteins and provide a support for their clinical validity as cancer targets. More specifically, this article reviews recent advances in clinical targeting of these malignancies by two types of growth factor/receptors, namely transforming growth factor-β (TGF-β), and EGFR subfamily of tyrosine kinase receptors including ErbB2. Overexpression of these proteins has been demonstrated in patients with cancer progression and correlated with poor prognosis, increased frequency of metastasis and death. In addition, EGFR and ErbB2 inhibitors have been used in targeted therapy of lung and breast cancer, respectively. Recent investigations of lung cancer have uncovered that EGFR inhibitors have their greatest effect in patients with EGFR somatic mutations thus raising a possibility that EGFR mutations may be a molecular predictors of sensitivity to EGFR inhibitors.

KEY WORDS: *Tumor Markers, Biological; Carcinoma; Disease Progression; Receptor, Epidermal Growth Factor; Oncogene Proteins; Transforming Growth Factor beta*

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INTRODUCTION

Cancer is a challenging disease for medicine due to a genetic heterogeneity that ultimately dictates clinical behavior. Over the past few years, the study of genomics has embarked on developing gene expression-based classifications for tumors - an initiative that promises to revolutionize cancer medicine (1). Genomic classifications from microarrays have now been developed for many malignancies, including breast, lung, prostate, brain, ovary, gastric, leukemia, and lymphomas. It is of interest to note that, in breast (2), lung (3) and prostate (4) cancer, the major signatures that appear include oncogenes, oncoproteins, and other conventional biomarkers obtained from existing paradigms (2-4).

Cell proliferation and growth are regulated by a complex network of growth factor- and growth inhibitor-initated signal transduction pathways. The disruption of these signaling pathways, through genetic, epigenetic, or somatic alterations, is a major area of cancer research. Increasing evidence indicates that oncogenic activation of growth factors and their receptor proteins occur through mutations (oncoproteins) that lead to constitutive activation of the signaling pathways (5), thus providing the grounds for putative prognostic marker(s) and potential target(s) for treatment of various cancers. Present communication focuses specifically on aberrations of growth factor/proteins (oncoproteins) and their role in clinical and experimental oncology. In an effort to organize this text, an attempt was not made to cover every growth factor investigated as a potential cancer biomarker. Rather, this presentation summarizes state-of-the art for TGF-β and EGFR, the growth factor/receptors that are being extensively investigated for potential clinical applicability. In addition, significant presence of these oncoproteins in the shortlists of genomic molecular markers of breast, lung, and prostate cancer has been observed thus supporting their clinical validity as cancer targets (2-4).

Genomic molecular markers for cancer diagnostics

The most comprehensive genomic signatures have been uncovered in breast cancer (BC) where significant biological and clinical molecular classifications have been made using several different microarray platforms and with tumors of different stages (2). Table 1 reveals genomic classification of BC based on the appearance of two major clusters designated as luminal and basal with respective shortlists of the most significant overexpressed genes. The luminal class of tumors, exhibiting a molecular signature resembling the luminal cells of the breast duct, has been further subdivided into two different luminal clusters with either a favorable prognosis (luminal A) or a less favorable prognosis (luminal B/C). The basal class of tumors, on the other hand, shared genes expressed by basal myoepithe-

lial cells (2). The benefit of providing general biological classifications, such as luminal and basal, is to avoid confusion from naming a tumor class after a single marker that is promiscuous in expression.

Table 1. Comparative classification of clinical breast cancer types based on genomic molecular markers versus conventional prognostic markers as described by Robinson et al (2)

#	Genomic molecular markers: Cluster type	Risk	Gene expression signature	Conventional biomarkers
1	Luminal A / B/C	↓ / ↑	*ER*, PR, KRT8, (TGF-β), XBP1, TFF3, HNF3A...*	ER/PR positive
2	Basal	↑	*KRT5, KRT17,* ***TGF-β****, FABP7, KIT, MYC...*	ER/PR negative
3	17q21 region amplicon	↑	***ERbB2****, GRB7, RAS...*	**ERbB2 positive**

*Abbreviations: ER, estrogen receptor; PR, progesterone receptor; KRT8, keratin 8; TGF-β , Transforming Growth Factor-β; XBP1, X-box binding protein 1; TFF3, trefoil factor 3; HNF3A, hepatocyte nuclear factor 3; FABP7, fatty acid binding protein 7; KIT, c-kit; MYC, myc; RAS, ras

To date, the ERbB2 class presented in Table 1 is not as robustly defined by genomics as the other groups and the nomenclature is somewhat inconsistent. Amplification of the 17q21 region and expression of the *ERbB2* gene is currently the best indicator for this group of tumors (2). Reassuringly, as illustrated in Table 1, the dominant biological features identified by genomics are consistent with tumor classification already clinically used for risk stratification. Corresponding markers routinely used in BC diagnostics are two hormonal receptors (ER and PR) and oncoprotein ErbB2 (also known as HER-2/neu). The presence/absence of these proteins, commonly scored using immunohistochemistry, currently forms the basis of a rudimentary molecular classification system including: hormonal receptor-positive, hormonal receptor-negative, and ERbB2-positive tumors (Table 1). These classes have been integrated into the diagnosis and treatment of BC patients and help stratify the risk of recurrence.

Table 2. Shortlist of genomic molecular markers versus clinically used conventional biomarkers of lung and prostate cancer as described by Kaminki et al (3) and Welsh et al (4), respectively

Malignancy	Gene expression signature	Conventional biomarkers
LUNG	***EGFR****, cyclin D1,* ***TGF-β****, c-Myc, mutated k-ras, BCL2...*	**c-kit, EGFR**, SCF, CK-BB, CEA, ...
PROSTATE	*PSA, KRT8, KRT18,* ***TGF-β****, IGFBP-2, IGFBP-5, MIC-1, hepsin...*	PSA, **TGF-β**, IL6SR...

* EGFR, Epidermal growth factor receptor; TGF-β , Transforming Growth Factor-β; PSA, Prostate Specific Antigen; KRT 8, keratin 8; IGFBP-2, Insulin growth factor binding protein 2; Il6SR, interleukin 6 soluble receptor; SCF, stem cell factor; c-kit, a tyrosine kinase (CD117); CEA, carcinoembryonic antigen; CK-BB creatine kinase isoenzyme BB; MIC, microphage inhibitory cytokine.

Shortlists of the most significantly expressed genes versus conventional prognostic markers for lung (3) and prostate (4) cancer are illustrated in Table 2. Taken together, the described data (Table 1,2) demonstrate that phenotypic changes in carcinogenesis are associated with altered expression levels of multiple genes. Thus, the focus on a single gene as a disease marker is almost futile when compared to the use of multiple molecular markers accessible through genomics. In this context, the remaining part of this presentation will focus on oncoproteins, including TGF-β and EGFR, and their complementary role in a putative panel of multiple molecular markers to be used in cancer diagnostics.

Why is TGF-β intriguing as a cancer target?

Significant progress has been made in the last decade by many investigators to identify aberration of transforming growth factor-β (TGF-β) in cancer pathology (6). This growth factor is intriguing due to its numerous and often opposing cellular functions. It acts as a tumor suppressor and a tumor promoter, as an inhibitor and stimulator of cellular proliferation, apoptosis, and angiogenesis. The TGF-β signaling pathway's elucidation has shown it to be very complex and involves a TGF-β ligand, three types of TGF-β receptors (TβRI, TβRII, and TβRIII) and four TGF-β intracellular transducers (Smad 2,3,4,and 7). Many scientists (6,7), including ourselves (8-14), have postulated that members of the TGF-β signaling pathway may be good candidates for prognostic or predictive markers for cancer patients. With the discovery that TGF-β was a potent growth inhibitor of epithelial cells, and the identification of inactivating mutations within the TGF-β signaling pathway in solid tumors, it became clear that TGF-β signaling is a tumor suppressor pathway for early stages of cancer. However, in advanced cancer stages overexpression of TGF-β has been observed and it has been associated with poor patients' prognosis and increased frequency of metastasis (6). Increased TGF-β levels are found in plasma of patients with invasive prostate and breast cancer (7), and in serum of patients with colorectal cancer, hepatocellular carcinoma, lung cancer, and metastatic melanoma (6). We have previously determined, for the first time, significantly elevated plasma TGF-β levels in prostate cancer (PC) patients with invasive disease (9). These findings have lead us to propose that plasma TGF-β concentration may be a new tumor marker attributed to the presence of invasive PC cells that may be used in the prognosis of PC (9). Subsequently, confirmed by many laboratories, this marker has been proposed as one of the components of PC diagnostic multiplex panel. Indeed, a biomarker panel containing PSA, TGF-β, and IL6SR has been recently utilized to develop a prostate cancer nomogram to predict the disease regression after radical prostatectomy (15). By extending this line of research to breast cancer, we have recently confirmed and extended the evidence for a significantly elevated plasma TGF-β levels in advanced BC patients with a poor prognosis (12). Moreover, we have observed that in postmenopausal patients this elevation was associated with ER/PR negative status and decreased probability of survival (14). Although the complete molecular mechanism of action and the role of TGF-β in cancer progression still remains to be elucidated, current findings suggest that selective cancer-specific mutations are responsible for the observed overexpression in various malignancies. Loss of TβRII expression and selective mutations of TfIRII have been observed in renal and colon cancer, respectively (6). In addition, a TGF-β polymorphism (T29-C) has been associated with higher serum TGF-β levels and with an increased risk in advanced breast cancer (16). In terms of targeted therapy, attempts to block the effects of excessive TGF-β activity have been tried involving agents that inhibit TGF-β binding to its receptors. They include natural TGF-β inhibitors (e.g., decorin), neutralizing TGF-β antibodies, soluble extracellular domains of the receptors, and small molecule inhibitors of TβRI kinase activity (6). Further research, in this rapidly progressing field will likely lead to new diagnostic marker(s) and therapeutic strategies.

EGFR AS A CLINICAL TARGET IN CANCER

The most frequently implicated receptors and growth factors in human cancer are members of the EGFR subfamily of tyrosine kinase receptors (2,3). The type I subfamily includes EGFR (ErbB1), HER-2/neu (ErbB2), HER-3 (ErbB3), and HER-4 (ErbB4). These receptors share a common molecular architecture including a large glycosylated extracellular ligand-binding domain, a single hydrophobic transmembrane domain, and a cytoplasmic tyrosine kinase domain (17). In breast cancer, *ErbB2* (also known as HER-2 or neu) is overexpressed in 20-30% of all BC patients and is associated with a high risk of relapse and death (18). One of the main reasons for the clinical utility of the tissue measurement of ErbB-2 is the selection of patients with invasive BC for trastuzumab (Herceptin) therapy. This agent is a humanized monoclonal antibody that has specificity for extracellular domain of the receptor (18). In lung cancer, overexpression of EGFR has been reported to be present in over 50% of cases in several series (19). Moreover, EGFR has been used as a therapeutic target in clinical trials involving the tyrosine kinase inhibitors (TKIs), including Gefitinb and Erlotinib that inhibit the intracellular tyrosine kinase domain by interfering with autophosphorylation by adenosine triphosphate (ATP). Initially, therapeutic inhibition of EGFR by TKIs was demonstrated in only 10% to 20% of all lung cancer patients (20). Several investiga-

tions over the last two years have uncovered that mutations in EGFR underlie the sensitivity to EGFR inhibitors. These mutations are somatic and found only in lung tumor tissues (19). They have been identified only in the tyrosine kinase domain encoded by exons 18 to 24 of the EGFR gene and include missense mutations with changes in the amino acids in exons 18 and 21, several overlapping deletions in exon 19, and small in-frame insertions in exon 20. The most common EGFR mutations include the exon 19 deletion (61%) and the missense mutation (24%). Although the mechanism by which the mutations in the EGFR tyrosine kinase domain render the receptor more sensitive to the effects TKIs is not yet defined, these findings suggest that EGFR mutations may serve as molecular predictors of sensitivity to EGFR inhibitors. Currently, EGFR mutation detection is becoming clinically available and is being incorporated into clinical treatment decisions and into the design of ongoing clinical trials (19). Furthermore, despite the remarkable initial success in the systemic treatment by TKIs in advanced lung cancer patients with EGFR mutations, a substantial proportion of these patients will ultimately develop disease regression. The mechanism underlying such acquired resistance to EGFR TKIs is beginning to be understood involving a common secondary mutation, a substitution of methionine for threonine at position 790 (T790M), only in the recurrent tumor specimens. The bulkier methionine residue in the T790M mutation results in a steric hindrance that likely prevents TKIs from inhibiting EGFR phosphorylation (19). These findings should spur the development of second-generation EGFR inhibitors and guide the use of such agents to patients with this specific mechanism of acquired resistance.

CONCLUSION

The genetic signatures presently available using microarrays indicate that the expression profiles of a relatively small number of cancer-related genes may provide a molecular means of identifying clinically important molecular targets and biomarkers not identified before. Experimental approaches are required to combine data obtained from existing paradigms and those of tumor profiling by genomic analysis to link complementary aspects of cancer biology. The overexpression or increased function of many oncogenes, including *c-Myc*, mutated *K-ras*, *EGFR*, *TGF-β*, etc., has been implicated in the pathology of various cancers. Reassuringly, the gene expression signatures identified by genomics and proteomics include TGF-β and EGFR oncoproteins thus provide a support for their clinical validity as cancer targets.

Although TGF-β and members of the TGF-β signaling pathway are being evaluated as prognostic or predictive markers for cancer patients, a diagnostic role of TGF-β has not been established yet. Ongoing advances in understanding the TGF-β signaling pathway will enable targeting of this pathway for the chemoprevention and treatment of human cancer. On the other hand, new mutations of EGFR signaling pathway responsible for both sensitivity and resistance to EGFR inhibitors of lung cancer patients are being discovered on an ongoing basis. This topic is clearly interesting and important subject of further investigations and requires demonstration of the clinical significance of different EGFR mutations. Furthermore, the methods currently used to detect different EGFR mutations by DNA sequencing are time consuming, complicated and costly. While microarray technologies are emerging, validation of these techniques will be required. The combined information revealed by these studies will identify molecular determinants involved in cancer diagnosis, prediction of clinical outcome, and response to therapeutic intervention.

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