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The promise of biomarkers for personalized renal cancer care

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Biomarkers hold promise for personalizing renal cancer care by potentially identifying patients most likely to benefit from novel therapies. Porta and colleagues report that among subjects with advanced renal-cell carcinoma receiving sunitinib treatment, those with above-normal levels of the circulating biomarkers vascular endothelial growth factor and neutrophil gelatinase-associated lipocalin displayed significantly lower progression-free survival times. This Commentary reviews the current status of these biomarkers with respect to methodological issues, specificity, and biological plausibility.

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The era of personalized medicine is well upon us. Ushered in by the remarkable genomic and proteomic advances in our understanding of health and disease, personalized medicine promises a more precise determination of disease predisposition, diagnosis, and prognosis, earlier preventive and therapeutic interventions, a more efficient drug development process, and a safer and more fiscally responsive approach to medicine.

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Biomarkers are the essential tools for the implementation of personalized medicine. Broadly defined as characteristics that are objectively evaluated as indicators of normal biological processes, pathogenic processes, or pharmacologic responses, biomarkers have already revolutionized several facets of medicine. Particularly in the field of oncology, specific biomarkers such as HER2 (erbB2) and EGFR now allow us to individualize therapies for patients with breast and colorectal cancers. However, we have only begun to scratch the tip of the proverbial iceberg—as medical breakthroughs continue to identify novel agents for malignancies that are potentially effective but also inevitably toxic and expensive, biomarkers will be indispensible for patient selection, risk stratification, and prognostication.

Consider the case in hand: renal-cell carcinoma (RCC), the most common kidney cancer in adults and clearly the most lethal of all advanced genitourinary tumors. Fortunately, an improved understanding of the underlying molecular biology has begun to identify novel targeted therapies. In kidney tubule cells, the product of the *VHL* tumor suppressor gene normally inhibits the production of hypoxia-inducible factors such as vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF). Inactivation of the *VHL* gene, as occurs in the majority of clear-cell carcinomas (the predominant type of renal-cell carcinoma), results in overexpression of these factors, and stimulation of the cognate VEGF and PDGF receptors, respectively. Persistent activation of the receptors' intrinsic tyrosine kinase results in signaling through cellular pathways that stimulate growth, proliferation, and angiogenesis and inhibit apoptosis, thereby resulting in metastatic RCC.[1] Sunitinib, an orally active agent that inhibits the receptor tyrosine kinases VEGFR types 1 and 2 and PDGFR-α and PDGFR-β, has shown promise in prolonging progression-free survival in some patients with advanced RCC.[2] However, individual responses have varied unpredictably, side effects are myriad, and overall mortality has remained largely unabated. Herein lies the dilemma—how do we personalize this novel therapy to best identify the subset of patients who are most likely to benefit from it?

Porta and colleagues[3] (this issue) have appropriately called on biomarkers to tackle this question. In 85 subjects with advanced RCC who were treated with sunitinib, the authors have compared the ability of an existing clinicopathologic scoring system (Motzer criteria) versus serum biomarkers at enrollment as predictors of progression-free survival. In their cohort, the baseline concentrations of both chosen biomarkers, VEGF and neutrophil gelatinase-associated lipocalin (NGAL), were found to correlate with progression-free survival, whereas the Motzer score did not. When subjects were dichotomized to normal versus high circulating biomarker levels, those with values above a threshold (determined

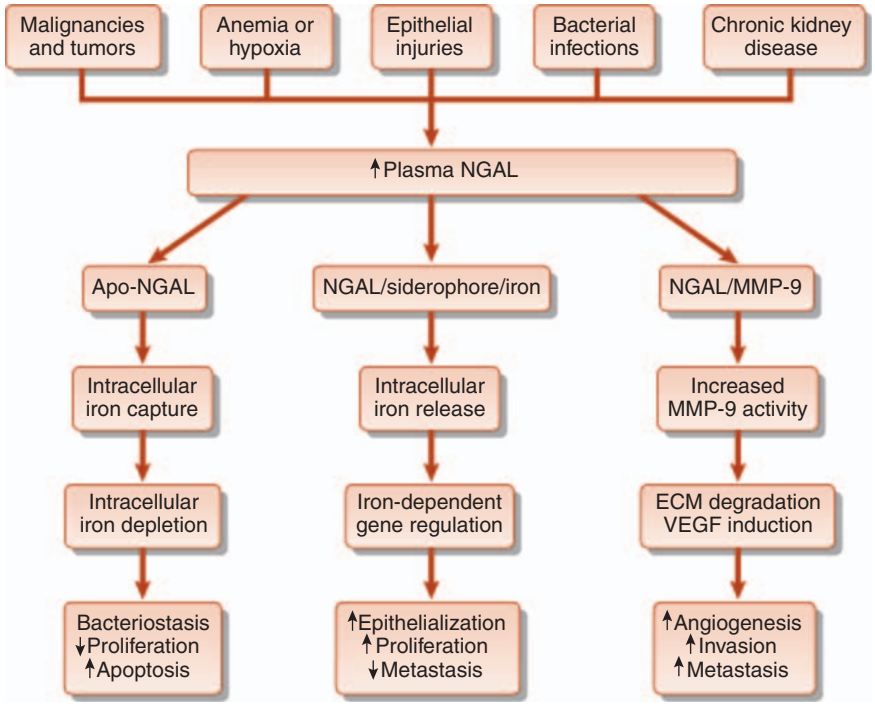

Figure 1 | Multiple factors may determine plasma NGAL concentrations. The cellular role of neutrophil gelatinase-associated lipocalin (NGAL) may be largely dependent on the type of molecule it is complexed with. 'Apo-NGAL' denotes NGAL that is devoid of siderophore or iron. ECM, extracellular matrix; MMP-9, matrix metalloproteinase-9; VEGF, vascular endothelial growth factor.

by normal ranges according to the manufacturers of the research-based ELISAs used) were found to have significantly diminished progression-free survival rates.

Like all good pilot studies, the report of Porta *et al.*[3] raises several questions that will need to be answered in future studies. First, an ideal biomarker should be easily measured, preferably with the use of standardized clinical platforms. Such a platform is now available for plasma NGAL,[4] but VEGF measurements continue to rely on research-based assays, which have been fraught with inconsistencies depending on the method of sample collection, processing, and interpretation.[5] The authors' choice to measure VEGF in the serum instead of the plasma may be particularly problematic. VEGF is normally stored in circulating platelets, and released during the clotting process. Therefore, serum VEGF concentrations may reflect blood platelet degranulation *in vitro* rather than VEGF synthesis by peripheral tissues. Second, the diagnostic accuracy of a biomarker is best assessed by the construction of receiver operating characteristic curves and determination of the area under the curve.[6] Such analysis can also provide biomarker concentration cutoffs that maximize the sensitivity and specificity to predict an outcome. Third, serial measurements of the biomarkers during the course of therapy may provide additional predictive information in comparison with a single baseline biomarker measurement.

Fourth, and perhaps most importantly, an ideal biomarker should be specific and attributable to a known mechanism. In RCC, the choice of VEGF is biologically plausible, based on the well-documented overproduction of this cytokine by clear-cell carcinoma cells with *VHL* mutations. However, there exist issues of biomarker specificity—increases in circulating VEGF levels accompany any angiogenic process, including diverse tumors, hypoxic states, anemia, diabetes, wound healing, and pulmonary hypertension, to name a few. The fact that sunitinib itself increases plasma VEGF levels further complicates the use of this biomarker once pharmacotherapy for RCC has begun.

What about the specificity of NGAL as a potential circulating biomarker in RCC? In the past few years, NGAL has advanced to center stage as an early predictive biomarker of acute kidney injury[7] as well as chronic kidney disease.[8] However, the precise sources of plasma NGAL in these conditions have remained controversial. Human NGAL was originally identified as a 25-kDa protein covalently bound to matrix metalloproteinase-9 (MMP-9) from neutrophils. NGAL is expressed at low levels in several human tissues but is markedly induced in injured epithelia, including the kidney, colon, liver, and lung. The overexpressed NGAL protein released into the circulation may contribute to the systemic pool. In addition, NGAL is an acute-phase reactant that may be released into the plasma from activated neutrophils, macrophages, other immune cells, and even atherosclerotic plaques. Also, both anemia and hypoxia can upregulate hepatic NGAL synthesis with resultant increases in plasma NGAL levels. Furthermore, any decrease in glomerular filtration rate resulting from acute or chronic kidney disease (or nephrectomies in the case of RCC) would be expected to decrease the renal clearance of NGAL, with subsequent accumulation in the systemic circulation. All these potential variables may diminish the specificity of NGAL as a biomarker in advanced RCC (Figure 1).

With respect to biomarker plausibility, it is instructive to ponder the potential roles that NGAL could play (Figure 1). Teleologically, NGAL constitutes a critical component of innate immunity to exogenous bacterial infections. Siderophores are prominent ligands for NGAL, and bacteria produce siderophores to shuttle iron from the extracellular space. The siderophore-chelating property of NGAL therefore renders it a bacteriostatic agent.[9] However, in the context of an injured organ such as the kidney, the biological role of NGAL induction is one of marked preservation of function, attenuation of apoptosis, and an enhanced proliferative response.[10] This protective effect is dependent on the chelation of toxic iron from extracellular environments, and the regulated delivery of siderophore and iron to intracellular sites. And finally, NGAL is markedly induced in a number of human cancers, where it often represents a predictor of poor prognosis.[9] The *NGAL* gene (also known as *LCN2*, for lipocalin 2) is known to be induced by a number of tumor-promoting agents, including SV40 and polyoma virus, phorbol esters, the

transforming factor *neu*, hepatocyte growth factor, retinoic acid, glucocorticoids, and nuclear factor-κB. The overexpressed NGAL protein binds to MMP-9, thereby preventing MMP-9 degradation and increasing MMP-9 enzyme activity. In turn, MMP-9 activity promotes cancer progression by degrading the basement membranes and extracellular matrix, liberating VEGF, and thus enabling angiogenesis, invasion, and metastasis. Paradoxically, recent studies in several tumor cell lines have shown that NGAL enhanced the epithelial phenotype, reduced tumor growth, and suppressed metastasis; this prosurvival activity of NGAL is mediated by its ability to bind and transport iron inside the cells.[9] Especially pertinent to the study by Porta *et al.*[3] are recent findings that (1) NGAL is induced by hypoxia and may indeed be driven by the pro-neoplastic hypoxia-inducible factor-1α (HIF-1α), which in turn is regulated by *VHL*; (2) NGAL can inhibit HIF-1α; and (3) NGAL can inhibit VEGF synthesis.

How does one reconcile the seemingly contradictory roles of NGAL in human biology? A potentially unifying hypothesis is offered in Figure 1. Efficient mechanisms have evolved for the intracellular uptake of NGAL via receptors such as megalin, and for intracellular trafficking via endosomes. The subsequent molecular path taken by NGAL may be largely dependent on the type of molecule it is complexed with. NGAL that is devoid of siderophore and iron (holo-NGAL) rapidly scavenges intracellular iron. The resultant intracellular iron depletion results in a decrease in the mammalian cell's proliferative ability and in induction of apoptosis. On the other hand, when NGAL is bound to siderophore and iron, there is a rapid release of iron with regulation of iron-dependent molecular pathways and downstream induction of proliferation and epithelial transformation. Finally, when NGAL is complexed with MMP-9 instead, there is enhancement of the active MMP-9 pool with resultant upregulation of MMP-9's well-known proangiogenic and proinvasive properties. Future studies aimed at further testing these hypotheses hold promise for advancing our understanding of tumor biology, and for potentially validating NGAL as a biomarker for personalizing renal cancer care.

DISCLOSURE

PD is a co-inventor on patent applications covering the use of NGAL as a biomarker of acute and chronic kidney diseases, and has received honoraria from Abbott Diagnostics and Biosite Inc.

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LECT2 amyloidosis

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LECT2 amyloidosis is the latest systemic type of amyloidosis to be described. It was discovered in patients with nephrotic syndrome and renal failure and is characterized by amyloid deposition in glomeruli, renal vessels, and interstitium. Clinical and pathological features of earlier phases of this type of amyloidosis have yet to be determined.

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In 1971 Glenner *et al.* reported the amino acid structure of monoclonal immunoglobulin light chain (AL) protein isolated from tissues of a patient with primary amyloidosis.[1] This was the first human amyloid protein to be chemically characterized. Structural characterization of human secondary amyloid protein (serum amyloid A (SAA)) was soon to follow in 1972,[2] and since then, eight additional proteins associated with human systemic amyloidosis have been identified, for a total of ten (Table 1). The latest of these amyloid proteins is leukocyte chemotactic factor 2 (LECT2), which, so far, appears mainly to cause renal amyloidosis.[3]

LECT2 amyloid was originally discovered in a patient who had nephrotic syndrome and slowly progressive renal failure over a number of years (Figure 1). Clinical evaluation and renal biopsy studies failed to determine the type of amyloidosis, and only when the patient developed renal-cell carcinoma, which necessitated nephrectomy, did amyloid-laden tissue become available for biochemical analysis. Without this chain of events and the inquisitive mind of the renal pathologist, it is unlikely that the correct diagnosis for this patient would have been made, and LECT2

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