Molecular Profiling of Gastric Cancer: Toward Personalized Cancer Medicine

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See accompanying article on page 868

Gastric cancer is the fourth most common cancer worldwide and the second leading cause of global cancer deaths. The prognosis of patients diagnosed with gastric cancer continues to be dismal, despite improving surgical and adjuvant treatment approaches, with a 5-year overall survival less than 25%. The high disease incidence and poor outcomes continue to make gastric cancer a topic of active clinical and basic scientific research. Notwithstanding these efforts, relatively little is known about the molecular changes that contribute to the development of this deadly disease and how they may influence outcomes and therapeutic results.

Gastric cancer can be considered a multifactorial disease because many inherited and environmental factors play a role in its carcinogenesis, including the genetic background of the host, infectious agents such as Helicobacter pylori, and dietary habits. Gastric cancer is a heterogeneous disorder that can be divided into at least the following two main histologic types on the basis of the Lauren classification: intestinal and diffuse types. These two types have distinct morphologic, clinical, and epidemiologic features and may also represent a different molecular mechanism of tumor development and progression.

The identification of germline E-cadherin (CDH1) mutations in families with hereditary diffuse gastric cancer provided early insight into the initial changes that promote gastric tumorigenesis and established CDH1 as a bona fide tumor suppressor. Subsequent studies indicated that, although germline CDH1 mutations are common in hereditary gastric cancer of the diffuse, but not intestinal, type, somatic mutations in CDH1 are frequently identified in sporadic gastric cancer of both major histologic subtypes. In addition, somatic epigenetic alterations, such as CDH1 promoter hypermethylation, are present in diffuse- and intestinal-type tumors and result in gene silencing. This epigenetic silencing of CDH1 gene expression represents the “second hit” in approximately 50% of hereditary diffuse gastric cancers, whereas the prevalence of CDH1 promoter hypermethylation in sporadic gastric cancers ranges in estimates from 11% to 46% depending on the histologic subtype.

Relatively small sample sizes and a focus on hereditary cancers or a single histologic subtype have hampered previous studies that evaluated the prevalence of CDH1 somatic mutations in gastric cancer overall. The current study by Corso et al evaluated a large set of familial and sporadic gastric cancers to provide a more comprehensive analysis of the prevalence of CDH1 somatic mutations and their correlation with patient survival. The authors analyzed 246 patients with sporadic (n = 174) or familial (n = 72) gastric cancer for CDH1 structural changes (either mutation or loss of heterozygosity) or epigenetic alterations (CDH1 promoter hypermethylation). The results indicated that, overall, 31% of gastric cancers carried CDH1 alterations, two thirds of which were epigenetic alterations and one third of which were structural alterations. Patients with CDH1 structural alterations displayed a poorer overall survival than in patients with either no changes or epigenetic changes only.

In addition, the authors report that CDH1 alterations (both structural and epigenetic) were found in 27.5% of intestinal-type and 38.4% of diffuse-type gastric cancers. Although these alterations were not associated with a worse overall survival in patients with diffuse-type tumors, there was a strong association between CDH1 structural alterations and poorer overall survival in patients with intestinal-type tumors.

The major finding by Corso et al that nearly one third of gastric cancers harbor somatic CDH1 alterations and those with structural alterations carry a poorer prognosis raises the question of whether somatic CDH1 alterations should influence the clinical management of gastric cancer. Although the findings in this study significantly clarify the extent of somatic CDH1 alterations in gastric cancer, there continues to be insufficient evidence to warrant regular CDH1-alteration analysis as a standard prognostic indicator or for predictive use in therapeutic decision making. However, the incorporation of this and other molecular features to more specifically characterize gastric cancers should certainly be part of the design of prospective studies of surgical and adjuvant therapy for this disease.

The role of epigenetic changes and tumor-suppressor silencing through promoter hypermethylation in human cancers has raised the specter of the use of demethylating agents such as 5-azacytidine as a therapeutic intervention. Large-scale changes in DNA methylation have been noted in the gastric cancer genome, and the CpG island methylator phenotype may predict a poor outcome independent from the tumor stage. A large number of genes that are suppressed by hypermethylation have been reported in gastric cancer in addition to CDH1, including the DNA mismatch repair genes, CDKN2A (p16), and MGMT (O6-methylguanine DNA methyltransferase). Whether therapeutic demethylation of some or all of these genes in gastric cancer may lead to tumor responses has yet to be tested. The use of demethylating agents has also been considered a potential chemoprevention strategy for carriers of the germline CDH1 mutation to slow or reverse the epigenetic second hit that inactivates CDH1 expression.
Although such an epigenetically targeted approach awaits additional investigation in gastric cancer, there is mounting evidence that somatic alterations, including copy-number variations in key oncogenes, such as amplifications of HER2, FGFR2, and MET represent viable treatment targets for which therapeutics are already approved or currently under investigation.

Individual molecular profiling of gastric cancers at a whole-genome level have revealed several known (e.g., p53, PTEN, and PIK3CA) as well as previously unreported (ARID1A) somatic gene mutations and pathway alterations. Of more immediate potential application, somatic copy-number aberrations have been found to be common in upper-GI cancers; 28% of gastric cancers have been shown to harbor amplifications in targetable membrane tyrosine kinases. The HER2 oncogene has been found to be amplified in from approximately 12% to greater than 20% of gastric cancers, and a phase III trial that investigated trastuzumab treatment of HER2-positive gastric cancers demonstrated an increased overall survival and response rate. Additional amplified genes that are currently being explored as therapeutic targets in the clinic include FGFR2, EGFR, and MET. Small molecules and therapeutic monoclonal antibodies are in development for each of these targets, and their role in gastric cancer as single agents and in combination with chemotherapy is being explored in ongoing phase I and II trials. Early results suggested that amplifications of each of these potential targets occur in from approximately 5% to 15% of gastric cancers. However, whether they are mutually exclusive or whether various combinations of these oncogenic targets will occur in individual tumors remains unknown. Furthermore, significant genetic heterogeneity has been shown to occur within and between primary cancers and individual metastatic sites. This heterogeneity is clearly the case for gastric cancers as well and will prove challenging for rationally designed targeted-therapy approaches.

Nevertheless, initial studies, such as the one by Corso et al7 in the current issue of Journal of Clinical Oncology, and others are defining the genetic and epigenetic landscape of gastric cancers. These studies suggest, as has been appreciated in breast cancer for sometime, that multiple genetic and molecular subtypes of gastric cancer exist beyond those defined histologically, and the genomic profiling of individual tumors may present unique treatment options for patients. As genomic analyses become an increasingly available option for the evaluation and identification of treatment targets in the tumor of an individual, clinical oncologists will have more therapeutic options at their disposal but will also be required to understand the genetics of their diseases. The goal of personalized cancer medicine is to understand the relevant genetic factors that underlie the particular tumor of an individual and tailor therapy to that individual. Such studies as the one by Corso et al7 and others discussed in this editorial will hopefully lead to improved outcomes by using drugs and therapies that target specific gene products and lead to synthetic-lethal interactions with aberrant cancer-associated pathways.

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Revealing the Molecular Mechanism of Gastric Cancer Marker Annexin A4 in Cancer Cell Proliferation Using Exon Arrays

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Abstract

Gastric cancer is a malignant disease that arises from the gastric epithelium. A potential biomarker for gastric cancer is the protein annexin A4 (ANXA4), an intracellular Ca²⁺ sensor. ANXA4 is primarily found in epithelial cells, and is known to be involved in various biological processes, including apoptosis, cell cycling and anticoagulation. In respect to cancer, ANXA4-overexpression has been observed in cancers of various origins, including gastric tumors associated with Helicobacter pylori infection. H. pylori induces ANXA4 expression and intracellular [Ca²⁺] elevation, and is an important risk factor for carcinogenesis that results in gastric cancer. Despite this correlation, the role of ANXA4 in the progression of gastric tumors remains unclear. In this study, we have investigated whether ANXA4 can mediate the rate of cell growth and whether ANXA4 downstream signals are involved in tumorigenesis. After observing the rate of cell growth in real-time, we determined that ANXA4 promotes cell proliferation. The transcription gene profile of ANXA4-overexpressing cells was measured and analyzed by human exon arrays. From this transcriptional gene data, we show that overexpression of ANXA4 regulates genes that are known to be related to cancer, for example the activation of hyaluronan mediated motility receptor (RHAMM), AKT, and cyclin-dependent kinase 1 (CDK1) as well as the suppression of p21. The regulation of these genes further induces cancer cell proliferation. We also found Ca²⁺ could regulate the transmission of downstream signals by ANXA4. We suggest that ANXA4 triggers a signaling cascade, leading to increased epithelial cell proliferation, ultimately promoting carcinogenesis. These results might therefore provide a new insight for gastric cancer therapy, specifically through the modification of ANXA4 activity.

Introduction

Gastric cancer is the second leading cause of cancer deaths worldwide and shows high prevalence in Asian populations. Although the incidence of gastric cancer is declining, the overall 5-year survival rate remains low [1]. Determining the most efficient gastric cancer therapies and developing early-stage diagnostic tools are important strategies in affecting clinical outcomes. The comprehensive investigation of the molecular mechanisms that underlie gastric carcinogenesis could provide assistance in developing useful therapeutic strategies for this disease.

Helicobacter pylori is a gastric pathogen and is the predominant etiological factor for gastric carcinogenesis. Approximately half of the world's population is infected with H. pylori, and more than 60% of gastric cancer patients have a history of H. pylori-positivity [2,3,4]. Recent studies have shown that H. pylori can induce both the proliferation of gastric cancer cells and mucosal inflammatory responses [5,6]. Thus, in order to investigate the molecular mechanisms underlying gastric carcinogenesis, it is necessary to investigate the role and mechanisms of H. pylori in gastric carcinogenesis.

Annexins are ubiquitously expressed in most organisms, including animals, plants, fungi and protozoa. It is associated with a variety of physiological functions [7]. Based on the structure of their conserved core domain, annexins are considered to be intracellular Ca²⁺ sensors and phospholipid binding proteins. They have been observed to stimulate membrane trafficking and vesicle aggregation in response to increased intracellular [Ca²⁺], [8,9]. In humans, annexins have been observed to have a range of cellular functions that have been implied in cytoskeletal organization, exocytosis, endocytosis, ion channel regulation, inflammation, apoptosis, fibrinolysis and coagulation [8]. Annexins are also considered to be involved in cancer, diabetes and inflammation [10]. Recently, more and more studies have emerged that implicating the involvement of annexins in carcinogenesis, as well as promoting proliferation [11,12], invasion [13] and metastasis [14,15]. However, the relationship between all members of the annexins family with cancer has not been characterized.

Annexin A4 (ANXA4) is a member of the annexins family associated with the digestive system. It is prominently expressed in epithelial cells [16]. Recent studies have shown that ANXA4 is considered to be a potential gastric biomarker based on its...