Critical Reviews in Oncology/Hematology 100 (2016) 141–146

Contents lists available at ScienceDirect

Critical Reviews in Oncology/Hematology

journal homepage: www.elsevier.com/locate/critrevonc

Translating gastric cancer genomics into targeted therapies

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Contents

ARTICLE INFO

Article history:
Received 18 September 2015
Received in revised form 30 January 2016
Accepted 15 February 2016

Keywords:
Gastric cancer
Molecular classification
Targeted therapy
Immunotherapy

ABSTRACT

Gastric cancer is a common disease with limited treatment options and a poor prognosis. Many gastric cancers harbour potentially actionable targets, including over-expression and mutations in tyrosine kinase pathways. Agents have been developed against these targets with varying success- in particular, the use of trastuzumab in HER2-overexpressing gastric cancers has resulted in overall survival benefits. Gastric cancers also have high levels of somatic mutations, making them candidates for immunotherapy; early work in this field has been promising. Recent advances in whole genome and multi-platform sequencing have driven the development of molecular classification systems, which may in turn guide the selection of patients for targeted treatment. Moving forward, challenges will include the development of appropriate biomarkers to predict responses to targeted therapy, and the application of new molecular classifications into trial development and clinical practice.

1. Introduction

Gastric cancer is the fifth most common cancer worldwide, and one of the leading causes of cancer-related mortality (International Agency for Research on Cancer, 2014). The treatment options for gastric cancer are limited, and patients invariably have a poor prognosis- patients with Stage 3 and 4 gastric cancer have 5 year overall survival rates of 9.2–19.8% and 4.0% respectively (National Cancer Institute, 2014). Despite recent breakthroughs in the use of targeted therapy in many other cancers, similar advances in gastric cancer have been slower. Part of the challenge arises from the heterogeneity of gastric cancers on a clinical, histologic and molecular level, which demands an individualized approach (Tan, 2015).

Historically, gastric cancer has been subdivided by histologic subtype via the World Health Organisation (WHO) or Lauren classifications, each with distinct clinical and epidemiologic features. The Lauren classification divides gastric cancers into intestinal and diffuse types, accounting for 54% and 32% respectively (Polkowski et al., 1999). Intestinal gastric cancers tend to be associated with

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http://dx.doi.org/10.1016/j.critrevonc.2016.02.007

environmental factors like Helicobacter pylori infection, occur in the antrum, and are often preceded by intestinal metaplasia. Diffuse gastric cancers on the other hand are more poorly differentiated, occur in younger patients, have a poorer prognosis and are found in inherited conditions. The WHO classification splits gastric cancers by their resemblance to metaplastic intestinal tissue (Dicken et al., 2005; Hu et al., 2012). More recently, the genomic study and characterisation of gastric tumours have given further insight into the pathogenesis of these cancers, and identified new potential therapeutic targets. This may pave the way for the development of personalised prognostication and treatment.

2. Molecular classification

The increasing efficacy and accessibility of sequencing has allowed multi-platform sequencing in large numbers, and in turn drives the development of classification systems based not on histopathology but on molecular features. The landmark Cancer Genome Atlas (TCGA) study performed sequencing of 295 gastric cancer samples on 6 different molecular platforms. Based on this, gastric cancer was clustered into 4 groups- Ebstein-Barr virus (EBV) positive (9%), tumours with microsatellite instability (MSI) (22%), genomically stable tumours (20%) and those with chromosomal instability (50%) (The Cancer Genome Atlas Research Network, 2014).

The EBV positive subtype, interestingly, showed a high level of non-silent *PIK3CA* mutations (80%), of which 68% were recurrent, as well as mutations in *ARID1A* (54%) and *BCOR* (23%). The rate of *PIK3CA* mutations in the other subtypes was 3–42%. There was also a high prevalence of DNA hypermethylation, particularly of the *CDKN2A* promoter, and amplification of the genes encoding immune checkpoint ligands PD-L1 and PD-L2. The MSI high subgroup, characterised by high levels of microsatellite instability without major chromosomal abnormalities, were enriched in tumours with hypermethylation, especially of the *MLH1* promoter, leading to *MLH1* silencing. The chromosomal instability subtype was associated with extensive somatic copy-number aberrations, and amplifications in genes involved in the RTK (receptor tyrosine kinase)-RAS pathway that lead to its activation. Finally, the genomically stable subtype, which lacked either chromosomal alterations or microsatellite instability, was high in *CDH1* and *RHOA* mutations.

These molecular analyses showed that each of the various subtypes had certain candidate therapeutic targets. The presence of *PIK3CA* mutations in EBV positive tumours suggest that these tumours may be particularly amenable to PI3K inhibitors. It was noted that the *PIK3CA* mutations that occurred in EBV positive gastric cancers were scattered over the gene, rather than concentrated over the kinase and helicase domains in exons 9 and 20, as was seen in the other subtypes. It remains to be seen whether these *PIK3CA* mutations truly have functional significance on the PI3K pathway. In addition, with new strides being made in immunotherapy, the prevalence of PD-L1 and PD-L2 over-expression in EBV positive tumours may also be of interest. In the genomically stable subtype, *RHOA* and *CLDN18* gene products are potential therapeutic targets, while in the chromosomal instability subtype, VEGF and other RTK amplifications highlight the possible role for RTK inhibitors like ramucirumab. MSI cases generally lacked targetable amplifications, although mutations in *PIK3CA, ERBB2, ERBB3, EGFR* and *ARID1A* were occasionally seen.

A similar undertaking by the Asian Cancer Research Group (ACRG) looked at 300 primary tumours, on which gene expression profiling, genome-wide copy number microarrays and targeted gene sequencing were done (Cristescu et al., 2015). In this study, gastric cancer was divided into 4 groups- MSS/EMT (microsatellite stable/epithelial-to-mesenchymal transition), which encompassed the outliers on the EMT distribution, MSI, MSS/TP53+, which had patients with intact TP53 activity, and MSS/TP53-, tumours which had functional loss of TP53. This population was unique in that long term follow up data was available. On a clinical level, these subtypes had prognostic value- with best prognosis in the MSI subtype, then MSS/TP53+, MSS/TP53- and then MSS/EMT. MSI tumours were more likely to be intestinal and diagnosed at an early stage, while MSS/EMT tumours were diffuse and more likely to recur. On a molecular level, the MSI subtype was confirmed to have hypermutation. Amplifications of *ERBB2*, *CCNE1* and *CCND1* tended toward mutual exclusivity in the MSS/TP53- subtype, which can be targeted by trastuzumab, CDK2 inhibitors and CDK4/6 inhibitors respectively. The MSS/TP53+ subtype showed a higher prevalence of mutations in *APC, ARID1A, KRAS, PIK3CA* and *SMAD 4*. In particular, sequencing revealed that of the 3 most common *PIK3CA* mutations, the ones in the MSI subtype tended to be H1047R mutations (A- > T), while in MSS tumours, E542 K and E545K mutations (G- > A) prevailed. These mutant *PIK3CA* proteins have been shown to cause oncogenic transformation in vitro, but it is not known yet which ones are more susceptible to PI3K inhibitors (Kang et al., 2005).

New genetic alterations and associations with particular tumour types continue to be identified, and contribute to our understanding of gastric cancer. For instance, *RHOA* hotspot mutations have been found to be common in diffuse (14.3%) but not intestinal-type tumours, with suggestions of a role as a driver of tumorigenesis. Other possible drivers that have recently been identified include *MUC6*, which codes for a mucoprotective mucin, *RNF43*, which negatively regulates WNT signalling, *CTNNA2*, involved in cell adhesion, and *GLI3* and *ZIC4*, both of which are involved in sonic hedgehog signalling (Wang et al., 2014). Recently, 5 recurrent fusion genes have been identified- one of them, *CLDN18-ARHGAP 26*, is seen in 3% of Asian Gastric cancers and is thought to contribute to the invasiveness of tumour cells (Yao et al., 2015).

These studies suggest that we should be moving towards molecular screening and classification of gastric cancers, to stratify them for treatment and prognostic purposes. Despite this, we are still some way from a consensus on the most relevant system.

3. Tyrosine kinase targets

Many of the promising molecular targets in gastric cancer are receptor tyrosine kinases (RTKs). Deng et al. profiled copy number alterations in gastric cancers and found that at least 37% of them harboured genomic alterations in RTKs that may be targets for agents that are currently available or under development. These included 9% of tumours with *FGFR2* alterations, 9% *KRAS*, 8% *EGFR*, 7% *HER2* and 4% *MET* (Deng et al., 2015).

3.1. HER2

HER2 is a transmembrane tyrosine kinase and a member of the epidermal growth factor receptor (EGFR) family, involved in the regulation of cell proliferation, adhesion, migration and differentiation. This occurs via heterodimerization with other members of the HER family, leading to activation of the RAS-MAPK and PI3K-AKT pathways. The *HER2* gene is located on chromosome 17q21 (Gravalos and Jimeno, 2008; Hudis, 2007). HER2 overexpression occurs in 15–30% of gastric cancers, and prevalence depends on the histology and location of the tumour- it is more common in the intestinal type (34% intestinal, 6% diffuse, 20% mixed) and in gastro-oesophageal junction tumours (32% in GEJ tumours vs 18% in gastric cancers) (Bang et al., 2010). HER2 positivity can be defined by protein expression on immunohistochemistry, and is obtained when there is strong membranous reactivity in >/ = 10% of cancer cells on surgical specimens or a cluster of five or more cells with