Novel Therapeutic Targets in Gastric Cancer

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ABSTRACT

Gastric Cancer **(GC)** is the fourth most common malignancy and the second leading cause of cancer deaths, accounting for 10% of global cancer mortalities. Despite the progress made in recent years, the prognosis for patients with advanced-stage GC remains poor. The list of FDA-approved drugs for GC is relatively small, and only two targeted therapies, namely Herceptin (trastuzumab) and Cyramza (ramucirumab), exist on the market. In the metastatic setting, chemotherapeutics are still the primary choice for treatment and result in Objective Response Rates **(ORRs)** of only 20-40% and median Overall Survivals **(OSs)** of 8-10months. However, a few of new targeted therapeutic agents are currently undergoing clinical trials; and some of these show clinical anti-tumor activity when used independently or in combination with chemotherapy. Emerging evidence revealed a number of genetic aberrations in GC, including receptor protein kinases (e.g. ERBB2, FGFR, MET), key transducers in phosphatidylinositol 3-kinase (PI3K)/AKT signaling, and cytoplasmic protein kinases (e.g. TNIK). In this paper, we review current advances in the identification and validation of new molecular targets, and discuss potential developments in targeted therapies for treatment of GC.

Keywords: Gastric cancer; RTK; Gene amplification; Gene mutations.

INTRODUCTION

Gastric Cancer **(GC)** is the second leading cause of death from cancer worldwide [1]. It accounted for approximately 8% of total cancer cases and 10% of annual cancer deaths worldwide [2,3]. Geographically, GC is more prevalent in developing countries, with most new cases and deaths occurring in China, Japan, and Korea. The current treatments for GC include surgery, chemotherapy, radiotherapy, and targeted therapy against HER2 (trastuzmab) and VEGFR (ramucirumab) [4,5]. Although the prognosis for GC has been improved significantly in certain nations, such as Japan and Korea, with national preventive screenings, the five-year survival rate remains poor. The genome-wide profiling of genetic aberrations in primary tumor samples from GC patients revealed a number of potential targets, such as FGFR, MET, and PI3K, for the development of next-generation therapies for GC [5]. Selective inhibitors of these potential targets are being developed at various clinical stages for the treatment of cancers, including GC (Figure 1). In this paper, we review the current understanding of genetic aberrations in GC and the functional characterization of them as potential therapeutic targets.

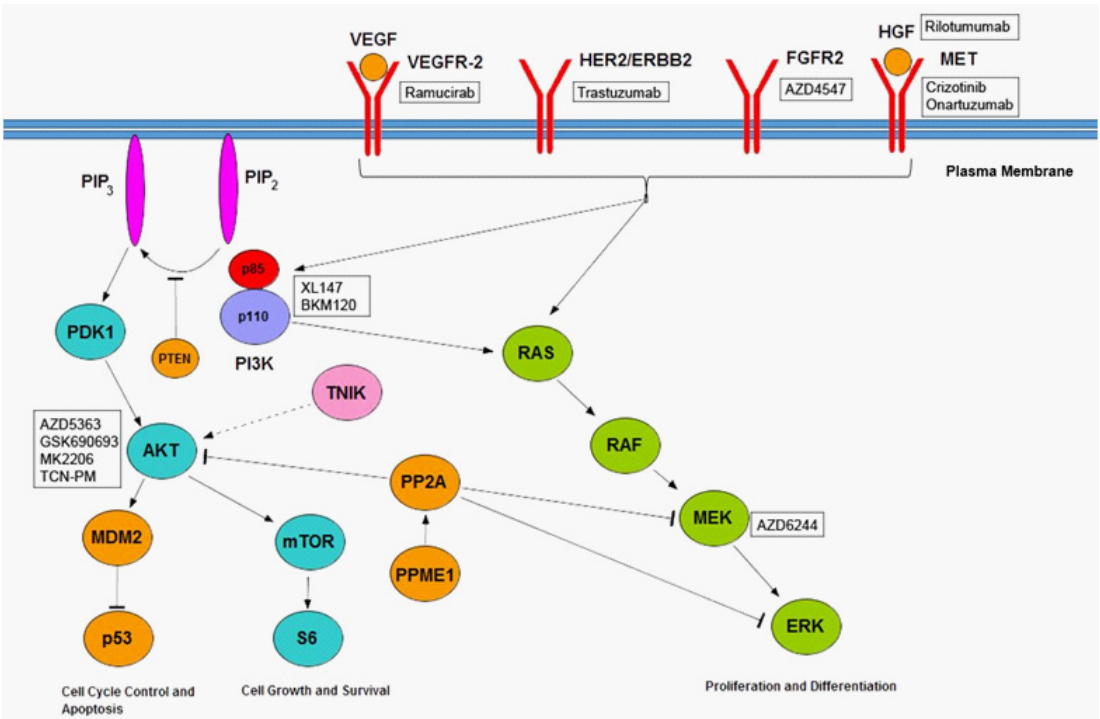

Figure 1: Schematic elucidation of activation of RAS/MAPK and PI3K/AKT signaling pathways, therapeutic targets and current targeted therapies at different stages of clinical development in gastric cancer.

MOLECULAR PROFILING OF GASTRIC CANCER

The genomic landscape of GC has recently been described by several groups through whole-genome analysis. This reveals a number of potential "driver" alterations, including somatic Copy Number Alterations **(sCNAs),** gene mutations, structural variants, epigenetic changes, and transcriptional changes involving mRNAs and noncoding RNAs **(ncRNAs).** These may serve as novel molecular targets for development of therapy to GC. Using a genome-wide array Comparative Genomic Hybridization **(CGH)** approach, Deng et al. [6] analyzed a panel of 233 GC samples and found 22 recurrent focal alterations. These include receptor tyrosine kinases (RTKs) and related genes (ERBB2, FGFR2, MET, KRAS), transcriptional factors (GATA6 and KLF5), and cell-cycle regulators (CCND1, CDKN2A, and MDM2). In additional to the previously identified amplifications such as EGFR, ERBB2 and CCND1, novel amplifications of the transcription factors GATA6 and KLF5, and deletions of PARK2, PDE4D, CSMD1 and GMDS were discovered. RTK alterations occurred in 37% of this patient cohort; the most frequently amplified RTK component was FGFR2 (9.3%), followed by KRAS (8.8%), EGFR (7.7%) and ERBB2 (7.2%).

To explore the spectrum of genetic aberrations in Chinese gastric cancers, we recently profiled 131 Chinese GC samples using the same array CGH technology [7]. 70 of the samples were further profiled by Affymetrix array analysis for global gene mRNA expression profiling. Consistently, a number of amplifications reported previously were also confirmed in our study [6]; these include ERBB2, MYC, MET, FGFR2, KRAS, GATA6, KLF5, EGFR, and EPHB3. Although the ethnic backgrounds of the patients were not specified in Deng's study, the concordant regions of genetic alteration and molecular targets identified in both studies highlight the reproducibility of our data. Besides the known amplifications, seven novel gene amplifications were identified: PPME1 (3.8%), TNIK (7%), CTSB (6.9%), PRKCI (6.1%), PAK1 (4.6%), STARD13 (4.6%), and ABCC4 (4.6%). Significant gene overexpression of PPME1, TNIK, KRAS, CTSB, PRKCI and PAK1, was also observed in the amplified cases. Among them, PPME1, TNIK, CTSB, PRKCI, and PAK1 are genes that encode either proteases or kinases, and are strong candidates for therapeutic targeting with small molecule inhibitors.

In additional to gene amplifications, gene mutations have also identified in GC. The application of Next Generation Sequencing **(NGS)** in GC led to identification of some novel potential driver mutations [8]. The mutations include genes related to genome integrity (TP53, BRCA2), chromatin remodeling (ARID1A), cell adhesion (CDH1, FAT4, CTNNA1), cytoskeleton and cell motility (RHOA), Wnt pathway (CTNNB1, APC, RNF43), and RTK pathway (EGFR, KRAS, PIK3CA and MLK3). These provide potential opportunities for personalized targeted therapy in GC.

Recently, as part of The Cancer Genome Atlas **(TCGA)** project, samples from 295 GC patients were comprehensively evaluated by single nucleotide polymorphism arrays, somatic copy-number analyses, whole-exome sequencing, mRNA sequencing, miRNA sequencing, array-based DNA methylation profiling, and reverse-phaseprotein arrays [9]. The data from this study yielded

a new molecular classification, which divides GC into four subtypes: Epstein-Barr Virus **(EBV)**-positive subtype which features recurrent PIK3CA mutations, extreme DNA hypermethylation, and amplification of JAK2, PD-L1and PD-L2; microsatellite unstable subtype with elevated mutation rates, including mutations of genes encoding targetable oncogenic signaling proteins; genomically stable subtype, enriched for the diffuse histological variant and mutations of RHOA or fusions involving RHO-family GTPase-activating proteins; and the subtype with chromosomal instability, characterized with aneuploidy and focal amplification of receptor tyrosine kinases.

Although the recent genome scale genetic profiling has yielded a large number of genetic aberrations with potential drugability for the development of new, targeted GC therapies, functional validation of them as clinically relevant "drivers" is crucial for successful drug discovery. In recent years, many groups, including our own, have validated a number of these potential targets by using biology and small molecule tools. In the following, we review the current statuses for the characterization and validation of each of these genetic aberrations as potential therapeutic targets. For ease of presentation, our review has been structured to summarize each molecular level independently.

VALIDATION OF GENE AMPLIFICATIONS AS NOVEL MOLECULAR TARGETS IN GC

FGFR2 Amplification

Fibroblast Growth Factor Receptor Family Members **(FGFR1-4)** belong to the RTK superfamily, which is involved in a diverse array of cellular functions including developmental regulation, mediation of cell proliferation and differentiation, angiogenesis, and tissue regeneration [10-12]. A linkage between genetic modifications and/or overexpression of FGFRs and tumorigenesis/tumor progression has been observed in breast, prostate, stomach, and hematologic malignancies [13-16]. In particular, abnormal activation of FGFR2 signaling has been linked with several types of human cancers, and somatic FGFR2 mutations have been reported in lung, gastric, and ovarian cancers [17-19]. FGFR2 amplification has also been associated with tumor cell proliferation and survival of GC cell lines [20]. To explore the potential for therapeutic targeting of FGFR2 amplification in GC, both aCGH and Fluorescence in situ Hybridization **(FISH)** assays were conducted to identify FGFR2 amplification in tumor samples patients with GC. FGFR2 gene amplification incidence rates of 4.5% and 7% were detected in cohorts of Chinese and Caucasian patients with GC, respectively, consistent with previously published reports [21,22]. The FGFR2 amplification was mutually exclusive with ERBB2 and c-MET amplifications in these patient samples [23].

The functional validation of FGFR2 in GC was conducted by two approaches in GC cell lines and Patient-Derived Xenograft **(PDX)** models: 1) pharmacologic modulation with the small-molecule inhibitor AZD4547, an orally bioavailable, highly selective, and potent ATP-competitive tyrosine kinase inhibitor of FGFR1-3; 2) shRNA knockdown of the FGFR2 gene. The results of

these studies show that FGFR2 gene amplification is an oncogenic driver in GC. GC cell lines with FGFR2amplification were extremely sensitive to AZD4547 *in vitro* with GI50 values of 3 to 5 nmol/L. Oral administration of AZD4547 or shRNA knockdown of FGFR2 resulted in rapid tumor regression in FGFR2-amplified models, markedly lowered phospho-FGFR2 levels, and suppression of downstream signaling throughphospho-PLCg, phospho-Erk, and phospho-S6. Thus, the preclinical data suggest that FGFR2 pathway activation is required for driving growth and survival of GCs carryingFGFR2 gene amplifications, supporting FGFR inhibitors, such as AZD4547, as potential therapeutic agents for the treatment of FGFR2-amplified gastric cancer. AZD4547 is currently in phase II clinical trials for GC. Other FGFR inhibitors, such as dovitinib (TKI258), Nintedanib (BIBF1120), Lenvatinib (E7080) also exist in the clinical trial stages and have high potential as therapeutic agents [24]. However, these inhibitors vary with regards to target specificity, and their efficacy in GI-originated, FGFR-amplified tumors remains to be tested.

PPME1 Amplification

Protein Phosphatase Methylesterase 1 **(PPME1)** is a protein phosphatase 2A (PP2A)-specific methyl esterase that negatively regulates PP2A by demethylation at the carboxy-terminal leucine 309 [25,26]. Emerging evidence shows that the upregulation of PPME1 is associated with poor prognosis in glioblastoma patients [27]. By performing an array CGH analysis to detect copy number changes, we have been the first to identify PPME1 gene amplification in 3.8% (5/131) of Chinese GC samples [28]. This PPME1 gene amplification was confirmed by FISH analysis and is correlated with elevated protein expression, as determined by Immunohistochemical **(IHC)** analysis. To further investigate the role of PPME1 amplification in tumor growth, shRNA-mediated gene silencing was employed. A knockdown of PPME1 expression resulted in a significant inhibition of cell proliferation and induction of cell apoptosis in PPME1-amplified human cancer cell lines SNU668 (GC) and Oka-C1 (LC), but not in non-amplified MKN1 (GC) and HCC95 (LC) cells. The PPME1 gene knockdown also led to a consistent decrease in PP2A demethylation at leu-309, which was correlated with the downregulation of cellular Erk and AKT phosphorylation [28]. Our data indicate that PPME1 could be an attractive therapeutic target for a subset of GCs.

TNIK Amplification

Traf2- and Nck-Interacting Kinase **(TNIK)** is one of the Germinal Centre Kinase **(GCK)** family members involved in cytoskeleton organization and neuronal dendrite extension [29]. Emerging evidence supports that TNIK is essential for activation of the Wnt signaling pathway in colon cancer proliferation [30,31]. TNIK gene amplification was identified in Chinese GC patients by aGCH assay at a rate of 7 % (8/106) [32]. Theses amplifications were confirmed by FISH analysis. RNA-interference-mediated silencing of TNIK resulted in significant inhibition of cell growth and induction of cell death in TNIK amplified, but not in non-amplified, cell lines tested. This selective sensitivity to the TNIK inhibition was also observed under the effect of a small molecule TNIK inhibitor. Furthermore, our data indicated that TNIK's role in GC growth was not dependent on

Wnt signaling, but rather was involved in AKT activation and cell autophage in GC. Together, our results suggest that TNIK is a novel therapeutic target in GC and TNIK amplification can be potentially used for patient selection [32].

PAK1 Amplification

P21-activated protein kinase (PAK1), a serine/threonine kinase, serves as the target for a number of small GTP binding proteins and has been implicated in a wide range of biological activities including cytoskeletal remodeling, cell motility, apoptosis and transformation [33].

In our recent study, PAK1 gene amplifications were identified in 5% of the GC samples by aCGH, which was further confirmed by FISH [34]. The amplification of PAK1 was confirmed by FISH analysis in an independent cohort of 111 Chinese GC patients, where PAK1 amplification was detected in 6% of cases. The PAK1 amplification was correlated with an increase in protein expression according to immunohistochemistry staining. Using shRNA-mediated knockdown, we found that depletion of PAK1 selectively inhibited the growth of PAK1-amplified GC *in vitro* and *in vivo* [34].

This result is consistent with previous data reported by Liu et al [35]. In that study, the expression levels of PAK1 GC tissues from 40 patients were quantified by western blot. Overexpression of PAK1 was associated with gastric tumor progression and metastasis. In addition, we found that knockdown of PAK1 expression significantly inhibited anchorage-dependent and anchorage-independent growth in GC cells, and markedly inhibited GC cell xenograft tumor growth. In conclusion, PAK1 may also be a potent prognostic marker and therapeutic target in gastric cancer.

CTSB Amplification

Proteases are critical in tumorigenesis by facilitating rapid cell cycling, local invasion, angiogenesis, and metastasis. Cathepsin B (CTSB), a member of the papain subfamily of lysosomal cysteine proteases, is involved in tumor cell invasion, metastasis, and angiogenesis [36]. Emerging evidence has indicated that CTSB is up-regulated in many cancer nodes and metastatic lesions, and functions to protect tumor cells from apoptosis [36]. Our aGCH screen for gene copy number variations in primary tumor samples found genetic amplification of CTSB in 13% (14/107) of Chinese gastric cancers, further supporting the tumorigenic potential of CTSB. These amplifications were confirmed by FISH analysis. Due to the lack of CTSB amplified GC cell lines; we could not conduct experiment to evaluate its role in GC. Instead, we explored the function of CTSB by using a pancreatic cancer cell line with CTSB amplification [34]. Down-regulation of CTSB by siRNA interference resulted in significantly reduced cell proliferation and increased cell death in the CTSB amplified ells, but not in the non-amplified cells. Therefore, aforementioned these amplified genes may represent attractive therapeutic target for drug discovery in GC. Interestingly, among the nine cases with CTSB amplification identified, three of them (30%) overlapped with ERBB2 amplification. Identification of CTSB/ERBB2 interactions would prove useful in identifying

tumorigenic mechanisms in these complex cases. In addition, overlap of these genetic aberrations could lead to CTSB-mediated anti-ERBB2 resistance, or ERBB2-mediated anti-CTSB resistance. Thus, preclinical models of tumor suppression efficacy for independent and combined targeted inhibitor treatments could yield insights for optimal therapeutic prescriptions.

MET Amplification

MET is a receptor tyrosine kinase and is involved in cell growth, survival and migration. In GC, either MET or its ligand, HGF, can induce aberrant levels of MET activation [37]. Preclinical evidence has suggested that MET signaling is essential for the survival of GC cells with MET amplification. Suppression of MET kinase activity with a MET TKI or by MET knockdown using RNA interference led to downregulation of MET signaling and apoptosis of GC cells with MET amplification, but not in the control cells without MET amplification. Consistently, significant anti-tumor activity of a small-molecule MET inhibitor was also observed in tumors harboring MET amplification *in vivo* [38-40]. These results suggest that MET is a promising drug target for GC.

Rilotumumab, a human monoclonal antibody that blocks the binding of HGF to MET, showed survival benefit in combination with chemotherapy agents in a Phase II clinical trial in GC patients with high MET expression [41]. However, all clinical trials for rilotumumab on MET-positive GC patients were recently halted. In RILOMET-1, a double-blind, randomized Phase III trial, rilotumumab was shown to have adverse effects on patient prognosis and morbidity when taken in combination with chemotherapy cocktail, ECX, (median OS=9.6mo; ORR=30%) than when ECX was administered alone (median OS=11.5mo; ORR=39.2%) [42]. Other MET-inhibitors have also met with disappointing results: a Phase III trial for onartuzumab demonstrated no significant effect on patient survival (median OS=11mo; ORR=46%) as compared to a placebo (median OS=11.3mo; ORR=41%) when administered with chemotherapy regiment mFOLFOX6. However, crizotinib, a small molecule MET-inhibitor approved by the FDA for use in EML4-ALK fusion-positive lung cancers, still holds promise. Preclinical studies have demonstrated significant anti-proliferative and apoptotic effects on MET-positive gastric tumors, both *in vitro* and *in vivo*, whilst having no effect on MET-negative GC. A phase I trial of crizotinib also suggested that tumors with MET amplification, strictly defined as having a MET/CEP7 ratio greater than 2.2 as determined by FISH, are potentially sensitive to MET TKI treatment. This suggests MET amplification identified by FISH might be a more relevant biomarker for patient selection to guide anti-MET targeted therapies [44].

VALIDATION OF NEW GENE MUTATIONS AS THERAPEUTIC TARGETS

KRAS Mutations

The KRAS protein is a GTPase and plays a key role in the RAS/MAPK pathway. It is primarily involved in regulating cell division, cell differentiation, and death. The abnormal activation of

RAS/MAPK signaling in cancer development has been well documented [45]. Recently, we profiled tumor samples from a cohort of Chinese GC patients and found KRAS mutations and amplification at rates of 6% (8/134) and 5% (6/100), respectively [34] Higher KRAS mRNA expression was observed in KRAS-amplified samples. Six of the KRAS-amplified tumors (100%) were from patients with high CIN (chromosomal instability), whereas four KRAS mutant tumors (50%) were from those with low CIN. The occurrences of KRAS amplification and mutation were mutually exclusive.

AZD6244 is a potent and selective MEK1/2 inhibitor and can suppress RAS downstream singling [46]. To understand the role of RAS aberrations in GC, we tested the responsiveness of a panel of human GC cell lines to AZD6244. Among the tested cells, seven of 10 KRAS-mutant cell lines responded to AZD6244, with GI50 values less than 1 nmol/L. In contrast, none of the four tested KRAS amplified cell lines responded to AZD6244 with a GI50 greater than 10 nmol/L [34]. The results suggest that GC patients with KRAS mutations, but not amplification, could benefit from agents targeting RAS singling.

ATM Deficiency and DNA Damage Response Pathway

Disruption of DNA repair genes by somatic mutations or epigenetic modifications has been reported in a variety of human cancers. Targeting DNA damage response pathways thus holds high potential as a therapeutic strategy [47]. Ataxia Telangiectasia Mutated **(ATM)** plays a critical role in cellular signaling in response to DNA double-strand damage, and its alteration is associated with the development and progression of several types of human cancers.

Recently, Kubota et al. reported that ATM protein expression levels vary greatly among GC cell lines [48]. ATM protein expression levels in GC cells were inversely correlated with their sensitivity to the PARP inhibitor, olaparib. Consistently, reduction of ATM kinase activity using a small-molecule inhibitor (KU55933) or shRNA-mediated depletion of ATM protein increased olaparib sensitivity in p53-inactivated GC cell lines. Thus, ATM is a potential biomarker to guide targeted therapy against PARP-1 in GC with p53 deficiency, and administration of agents targeting both ATM and PARP-1 could be a new combination strategy for treatment of GC. In a randomized phase II study involved 124 patients, although combination of olaparib with paclitaxel did not result in significant improvement in PFS (overall population: Hazard Ratio **[HR],** 0.80; median PFS, 3.91 v 3.55 months, respectively), this combination significantly improved Overall Survival **(OS)** versus paclitaxel control in both the overall population (HR, 0.56; 80% CI, 0.41 to 0.75; P = .005; median OS, 13.1 v 8.3 months, respectively) and the ATM-low group (HR, 0.35; 80% CI, 0.22 to 0.56; P = .002; median OS, not reached v 8.2 months) [49]. A phase III trial in this setting is under way.

PIK3CA Mutations and PI3K/AKT Pathway

Class I phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) is a heterodimer comprised of an 85 kDa regulatory subunit and a 110 kDa catalytic subunit. The latter subunit has three variants, of

which the alpha variant (PIK3CA, or the p110α protein) has been documented in a range of tumor types [50,51]. Normal activation of the PI3K/AKT pathway is critical for the proper regulation of cellular metabolism, growth, proliferation, motility and survival. Constitutive activation of the PI3K/AKT pathway is a common phenomenon in cancer and can be due to multiple mechanisms, including mutation of PI3KCA, loss or mutation of PTEN, or over-expression of receptor tyrosine kinases [50]. PI3KCA mutation is a common factor for dysregulation of the PI3K/AKT pathway, posing high tumorigenic risk.

Although the presence of PIK3CA mutations do not significantly affect overall prognosis in GC patients as compared to GC cases without PIK3CA mutations [52], direct gene sequencing and whole genome sequencing has detected PIK3CA mutations in 7.7% - 42% of GCs [52]. In addition, 80% of PIK3CA mutations are also localized to one of 3 major clusters – all of this result in an upregulation of PI3Kα activity. Many inhibitors, including ZSTK474 [53] and INK1117, target the PI3Kα isoform, and many others more widely target the PI3K/AKT/mTOR pathway; these drugs are undergoing various clinical trial phases. Since mTOR is one of the downstream effectors of PI3K and AKT, it is expected that inhibitors of mTOR will be more selective than targeted agents for the other two. Emerging studies have shown PIK3CA mutations to correlate significantly with patient responsiveness and outcome to these targeted treatments making it as a potential predictive diagnostic marker [54].

Due to the high prevalence of PIK3CA mutations in GC, they are commonly found in conjunction with other aberrations in genetic and proteomic expression. This can complicate analysis of tumorigenic mechanisms, and pre-clinical studies documenting the effects of PIK3CA interactions are important for diagnostic correlations. We recently developed a novel AKT kinase inhibitor, AZD5363, and demonstrated that HGC27, a cell line harboring both PI3KCA mutation and PTEN loss, displayed the greatest sensitivity to this AKT inhibitor in vitro and in vivo [55]. Disease linkage studies showed that PI3KCA activating mutations or PTEN loss were found in 2.7% (4/150) and 23% (14/61) of Chinese GC patients, respectively [56]. To elucidate the correlation between AZD5363 response and these prominent genetic alterations, we identified GC patients with both PI3KCA mutations and PTEN loss and created a panel of 20 GC cell lines from these samples. Subsequently, we investigated the effects of pharmacological inhibition of AKT on these tumors and were able to demonstrate that GC cells with PI3KCA mutations were selectively sensitive to AZD5363. We then tested the antitumor activity of AZD5363 in two patient-derived GC xenograft (PDGCX) models harboring either PI3KCA mutation or PTEN loss. Our data indicated that AZD5363 monotherapy treatment led to a moderate response in the PI3KCA mutant PDGCX model. Whilst monotherapy AZD5363 or Taxotere were ineffective in the PTEN negative PDGCX model, significant anti-tumor activity was observed when AZD5363 was combined with taxotere [56]. Our results indicated that PI3KCA mutation is an important determinant of response to AKT inhibition in GC. In addition, combination therapy using AZD5363 can overcome innate resistance to Taxotere in a PTEN loss PDGCX model. It is thus suggested that AKT inhibitors are an attractive option for treatment of a new segment of GC patients with aberrant PI3K/AKT signaling.

ERBB2 (HER2) Mutation

Genetic amplification of HER2 drives tumorigenesis and cancer progression in a subset of patients with GC (GC), and treatment with trastuzumab, a humanized HER2-neutralizing antibody, improves the overall survival rate of HER2-positivepatients [57]. However, a considerable portion of the patients do not respond to trastuzumab and the molecular mechanisms underlying the intrinsic resistance toanti-HER2 therapy in GC is not fully understood [58]. We performed whole-transcriptome sequencing on 21 HER2-positive tumor specimens from Chinese GC patients [59]. We identified three new HER2 fusions with ZNF207, MDK, or NOS2 in 21 HER2-amplified GC samples (14%; 3/21). Two of the fusions, ZNF207-HER2, and MDK-HER2, which are oncogenic, lead to aberrant activation of HER2 kinase. Treatment with trastuzumab inhibited tumor growth significantly in xenografts expressing MDK-HER2 fusion. In contrast, trastuzumab had no effect on the growth of xenografts expressing ZNF207-HER2 fusion, due to its inability to bind to trastuzumab [59]. Our results provide the molecular basis of a novel resistance mechanism to trastuzumab-based anti-HER2 therapy, supporting additional molecule stratification within HER2-positive GC patients for more effective therapy options such as anti-HER2 kinase inhibitors.

FUTURE DIRECTION

The implementation of genome scale profiling technologies (e.g. NGS, aCGH) has accelerated our understanding of genetic aberrations. These have greatly improved our ability to comprehensively map the molecular basis of GC, thus facilitating better identification of novel biomarkers and therapeutic targets (Figure 1). The functional validation of so-called “drivers” as distinguished from co-existing “passenger” genes remains the first step for advancing potential targets into drug discovery programs. However, due to inherent molecular and geographical heterogeneities in GC, discrepancies can exist between *in vitro* validation and clinical outcomes. The increased application of large scale patient derived xenograft (PDX) models for *in vivo* validation is expected to narrow the gap between preclinical and clinical studies. New combination strategies are also likely to arise as we develop our understanding of the molecular mechanisms underlying tumorigenesis. With more stringent, clinically relevant biomarkers and assays for patient selection, targeted therapy can become even more personalized. With all of these recent advances, prognosis for GC patients is expected to improve significantly in the near future.

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