

2015 Advances in Gastric Cancer

Individualized treatment of gastric cancer: Impact of molecular biology and pathohistological features

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Author contributions: Dittmar Y compiled and analyzed data and wrote the paper; Settmacher U reviewed the paper and provided valuable scientific input.

Conflict-of-interest statement: The authors declare no conflict of interest.

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Received: April 29, 2015
Peer-review started: May 7, 2015
First decision: June 25, 2015
Revised: July 23, 2015
Accepted: September 30, 2015
Article in press: October 10, 2015
Published online: November 15, 2015

Abstract

Gastric cancer is one of the most common malignancies worldwide. The overall prognosis remains poor over the last decades even though improvements in surgical outcomes have been achieved. A better understanding

of the molecular biology of gastric cancer and detection of eligible molecular targets might be of central interest to further improve clinical outcome. With this intention, first steps have been made in the research of growth factor signaling. Regarding morphogens, cell cycle and nuclear factor- κ B signaling, a remarkable count of target-specific agents have been developed, nevertheless the transfer into the field of clinical routine is still at the beginning. The potential utility of epigenetic targets and the further evaluation of microRNA signaling seem to have potential for the development of novel treatment strategies in the future.

Key words: Gastric cancer; Molecular biology; Targeted therapy; Personalized medicine; Signaling pathway

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Core tip: Advanced gastric cancer remains a frequent malignancy with poor prognosis despite multimodal treatment options. Surgery alone has been demonstrated not to be the optimal strategy and is predominantly limited to cases without distant metastases. About one half of gastric cancer patients cannot be cured. Due to its individual heterogeneity on the molecular level these tumors frequently do not respond to systemic treatment. The implementation of the growing knowledge about the molecular behavior of gastric cancer in the development or improvement of target-specific treatment strategies might be one of the major challenges for the next decades.

Dittmar Y, Settmacher U. Individualized treatment of gastric cancer: Impact of molecular biology and pathohistological features. *World J Gastrointest Oncol* 2015; 7(11): 292-302 Available from: URL: <http://www.wjgnet.com/1948-5204/full/v7/i11/292.htm> DOI: <http://dx.doi.org/10.4251/wjgo.v7.i11.292>

GENERAL CLINICAL ASPECTS

Gastric cancer is still one of the leading oncologic challenges due to its frequent occurrence as well as its poor prognosis^[1]. The ongoing improvement of surgical techniques and perioperative care over the past decades have not only extended the repertoire of treatment options with curative intent but also have contributed to the reduction of perioperative morbidity. Thus, currently about 50% of all gastric cancer patients can be treated curatively and the majority of these patients undergo the surgical treatment without severe complications^[2]. But still one half of all gastric cancer patients have to be regarded as palliative cases with no chance for long term survival and even the curatively resected patients face an overall recurrence rate of 50%^[3].

In view of this development it can be assumed that further evolvement of surgical treatment will not improve tumor-related survival substantially. The molecular biology of the individual tumor might be one important key to a better understanding of the disease and an advancement in the prognosis of gastric cancer patients.

The knowledge about the molecular biology of gastric cancer is of high interest for several reasons: (1) aberrations at the genomic as well as at the proteomic level might be useful as biomarkers for exact classification; (2) molecular markers may further improve and refine tumor staging; (3) knowledge about the individual molecular signature may enable a personalized and target specific treatment; and (4) molecular presentation of the tumor and target specific treatment may lead to an improved prognosis.

UNDERSTANDING OF THE MOLECULAR BIOLOGY: GENERAL CHALLENGES

The understanding of molecular biology of gastric cancer is crucial for the appraisal of its clinical behavior and to control the tumor growth with all its consequences. As in almost all other tumor entities the following characteristics may challenge the establishment of an effective treatment: (1) every individual tumor presents with a unique pattern of molecular variance, comparable with an individual fingerprint; (2) in a certain manner every tumor can be regarded as an autonomous organism which in fact means that tumors do not consist of a homogenous tissue mass but show a regional heterogeneity; (3) over time every tumor changes spontaneously in its molecular biological behaviour; and (4) every tumor reacts in a distinct manner to treatment attempts.

These aspects are basically important in un-targeted treatment approaches as the application of conventional cytostatic substances or surgery but are even more important for target-specific treatment strategies. In view of the multidimensional complexity of molecular tumor biology it becomes clear that it is unlikely to find "the single one agent" to achieve a safe and sustainable

tumor control.

CURRENT STATE OF THE ART IN MOLECULAR TARGETED TREATMENT

Growth factors, growth factor receptors and downstream features

Epithelial growth factor: To date, four different types of epithelial growth factor receptors (EGFR) have been identified, also called as ErbB1-4^[4]. Once activated, they form homo- or heterodimers and then become internalized within the cell. From there three different pathways (MAP-kinase pathway, STAT pathway and PI3K pathway) can be activated, subsequently leading to the transmission of the signal into the nucleus and specific regulation of gene expression by activated cyclinD1, iNOS, B-myb, COX2 and Aurora kinase 2. With the exception of ErbB2, in addition to the original epidermal growth factors multiple other ligands can bind and activate EGFR: transforming growth factor alpha, epiregulin, amphiregulin and β cellulin. ErbB2 in contrast, can not be activated directly by any growth factor, but can be heterodimerized by other members of the EGFR family^[5].

It has been reported that EGFR overexpression occurs in 60% to 70% of gastric cancer cases, however gene amplification seems to be rather uncommon^[6,7]. EGFR2 measured by fluorescence *in situ* hybridisation was detected in 22% of gastric cancers^[8,9], while it was more frequent in intestinal than in diffuse type gastric cancer according to the Lauren classification (32% and 20%)^[9,10]. EGFR overexpression in gastric cancer was related to poorer survival and poorer response to chemotherapy^[11].

Due to its central role in epithelial signaling as well as its biological properties EGFR became an interesting target for molecular-based treatment and thus there is now a remarkable variety of EGFR-targeted molecules available.

Three main target points have been proposed: the inactivation of the receptor, the stimulation of antibody-dependent cell cytotoxicity and the inhibition of the tyrosine kinase activity by multityrosin kinase inhibitors.

To date, seven monoclonal antibodies targeting EGFR are available: cetuximab, trastuzumab, matuzumab, panitumumab, nimetuzumab, perluzumab and T-DM1^[10].

Cetuximab inhibits the binding of EGF and TGF α to EGFR, furthermore it promotes the internalization of the receptor^[12]. The application of cetuximab is well established in stage 4 colorectal cancer (with k-ras wild type)^[13] and in several head and neck malignancies^[14,15].

Several phase 2 and 3 trials showed a positive effect of the administration of cetuximab combined with standard chemotherapy protocols as a first line therapy with response rates up to 58% and 69% in advanced gastroesophageal junction and gastric cancer (overall survival up to 9.5 mo)^[10,16]. In contrast, cetuximab in combination with cisplatin or irinotecan as a second line

therapy revealed only a marginal benefit on the overall survival (7.1 mo)^[17]. Moreover, cetuximab as a single-agent administration for second line therapy resulted in even lower impact on the overall survival (3.6 to 4 mo) with poor response (9%)^[18].

Cetuximab in combination with several cytostatic substances for neoadjuvant chemotherapy showed response rates up to 70%^[19,20].

Trastuzumab is known to have a broad variety of molecular effects: Binding to the extracellular part of the her-2/*neu* molecule and thus suppressing the intracellular localised tyrosine kinase activity, antibody dependent cell toxicity (ADCC)^[21], activation of natural killer cells, inhibition of angiogenesis and the phosphoinositol-3-kinase signaling pathway (PI3K) as well as cell cycle arrest^[22-24]. The administration of trastuzumab as adjuvant treatment has been approved for node positive breast cancer^[25].

The most important study with respect to gastric cancer is the ToGA trial. It has been shown that those patients who were positive for the her-2/*neu* receptor (22% of all cases) had a significant improvement in tumor response and overall survival when standard chemotherapy was combined with trastuzumab (47% vs 34%, 13.8 mo vs 11.1 mo)^[26]. An innovative and promising further development of trastuzumab, named T-DM1 is currently undergoing clinical testing. In the T-DM1 molecule the trastuzumab antibody is coupled to maytansine, a microtubule polymerization inhibitor which unfolds its effect after internalization of the antibody-receptor complex within the cytosol^[27].

Recently it has been published that *in vitro* the cytotoxic effect of trastuzumab on gastric cancer cell lines significantly increased when the cancer cells were pre-treated by incubation with reovirus serotype 3^[28].

Matuzumab is an IgG1 antibody with ADCC. Unlike cetuximab and nimotuzumab it is a fully humanized molecule. Unfortunately, it has been shown that combination treatment of matuzumab with cytostatic substances is not beneficial for overall survival and response rates^[29].

Panitumumab is an IgG2 antibody. It is routinely used in the treatment of metastatic colorectal cancer. The comparison of combined chemotherapy with or without panitumumab yielded disappointing results with a poorer outcome in the panitumumab group in terms of overall survival and overall response rate (8.8 mo vs 11.3 mo and 42% vs 46%, respectively). Surprisingly, in the subgroup of patients with severe rash the overall survival of patients who received panitumumab-including treatment was significantly improved (10.2 mo vs 4.3 mo)^[30].

Nimotuzumab is similar to matuzumab a fully humanized antibody, known to exhibit ADCC. There is some evidence in the literature that nimotuzumab in combination with cytostatic substances might be effective in squamous cell carcinoma of the esophagus and in glioma. To date, there are two studies available investigating the effect of nimotuzumab plus cytostatic

substances in metastatic gastric cancer. In one study, the overall response rate was improved (63% vs 50%) with similar progression free survival, the other study showed the progression free survival to be slightly improved with similar response rates (5.5 mo vs 3 mo)^[10].

Pertuzumab is an inhibitor of homo - as well as heterodimerization of the EGF receptor. Therefore, it seems to be reasonable to combine pertuzumab with different EGF receptor antagonists like trastuzumab. It is also known to exhibit ADCC. The administration of pertuzumab is approved for metastatic breast cancer^[31]. The combination of pertuzumab and trastuzumab seems to be effective in advanced gastric cancer with overall response rates up to 86%^[32].

Vascular endothelial growth factor: The recruitment of new blood vessels for the supply of the growing tumor with nutrients and oxygen is known to be one of the crucial steps in tumor progression, especially in the development of distant metastases^[33]. Although neoangiogenesis in the tumor environment and physiological angiogenesis partly have similar pathways there are remarkable differences in vessel architecture, vascular permeability as well as a different interplay of endothelial cells and perivascular cells. In this context, vascular growth factors play an crucial role. Vascular growth factors are expressed when tissue hypoxia is present. Several other changes can result in vascular endothelial growth factor (VEGF) up-regulation too, *e.g.*, low pH or silenced tumor suppressor genes like p53^[34].

To date, we know five important factors of angiogenesis: VEGF A-D and placenta derived growth factor. Furthermore, three targets for these growth factors have been detected: vascular endothelial growth factor receptor (VEGFR) 1-3. VEGFR2 seems to be the most important subtype. It is localized on the cell surface of endothelial cells and bone marrow derived endothelial progenitor cells^[35]. VEGFR2 binds to VEGF A, C and D, leading to activation of the PI3K signaling pathway as well as MAP kinase signaling pathway^[36]. Some of the most important down stream effects are the inhibition of apoptosis, the proliferation of endothelial cells and increased endothelial cell migration^[35]. The binding of the mediator molecule to its receptor is substantially increased in the presence of the co-receptors neuropilin 1 and 2. The application of these co-receptors as possible targets for molecular based treatment is currently under development^[37].

Overexpression of VEGF and its downstream molecules is common in numerous malignancies. Interestingly, Takahashi *et al.*^[38] already demonstrated in 1996 that VEGF is more frequently dysregulated in intestinal type than in diffuse type gastric cancer (36% and 16%, respectively). Two different antibodies targeting the VEGF signalling pathway have been shown to be effective and eligible in the treatment of advanced gastric cancer: Bevacizumab and ramucirumab.

Bevacizumab binds to VEGF-A and thus interrupts

the activation of VEGFR1 and VEGFR2^[33]. Whereas different phase 1 and 2 trials revealed promising effects of bevacizumab on gastric cancer progression, the results of phase 3 studies were disappointing. Although in the AVAGAST study overall median survival was slightly longer in patients who received bevacizumab plus standard chemotherapy, these results did not reach a statistically significant level (12.1 mo and 10.1 mo, $P = 0.1002$). Merely progression free survival was significantly longer in the intervention group (6.7 mo and 5.3 mo, $P = 0.0301$)^[39]. The subsequently performed AVATAR study did not show any benefit of treatment with bevacizumab in combination with standard chemotherapy as compared to standard chemotherapy only (median overall survival 10.5 and 11.4 mo, progression free survival 6.3 and 6.0 mo)^[40]. Based on these results bevacizumab currently is not routinely used in the treatment of advanced gastric cancer.

Ramucirumab is a competitive inhibitor of VEGFR2 with a 8fold higher affinity to the receptor as compared to natural ligands^[41]. Two phase 3 studies revealed ramucirumab to have positive effects on the containment of gastric cancer progression. The REGARD study investigated the impact of ramucirumab as a second line therapy on advanced gastric cancer. In comparison to the placebo group as well overall survival, disease control rate and overall response rate were significantly better (3.8 mo vs 5.2 mo, 49% vs 23%, 3.4% vs 2.6%). Interestingly, among male patients these effects were even more distinct^[42]. The RAINBOW study compared the outcomes after administration of paclitaxel with or without ramucirumab to a similar target audience. Overall survival and disease control rate both were better in the intervention group (9.6 mo vs 7.4 mo, 80% vs 64%)^[43].

In summary, currently ramucirumab seems to be the only one option to treat advanced gastric cancer with a VEGF-R specific antibody.

Platelet derived growth factor receptor: The Platelet derived growth factor (PDGF) family consists of 4 homodimers A-D and the heterodimer AB. Due to its dimeric structure it binds to receptor molecules which subsequently activate each other. Two different subtypes of PDGF receptors have been identified (alpha and β)^[44]. Under physiological conditions PDGF is released when platelets are damaged. Furthermore, PDGF signalling is known to play an important role in the embryonic development of kidney, blood vessels, lung and several components of the central nervous system^[45,46].

In several aspects the importance of the PDGFs as well as its corresponding receptors have to be regarded as being closely connected with the VEGF system. Whereas activation of VEGF signalling leads to recruitment of new blood vessels, one important downstream effect of PDGF signaling is the maintenance of microvessels. The regulation of the tumor environment - especially activities of fibrocytes and pericytes - as well

is partly realized by the PDGF signalling pathway^[46].

Up-regulation of PDGF signaling has been demonstrated for prostate cancer, breast cancer, lung cancer as well as colorectal cancer. In gastric cancer it has been shown that PDGF is frequently overexpressed in tumor cells whereas its corresponding receptor is overexpressed in several cell types of the microenvironment. It has been postulated that the tumor cell derived PDGF signal selectively leads to the up-regulation of PDGFR expression in environmental non-tumour cells^[46].

To date, there are no PDGF specific antibodies available for clinical use regarding gastric cancer.

Fibroblast growth factor: The fibroblast growth factor family consists of 23 molecule subtypes, targeting four different FGF receptor subtypes. In addition, several co-factors like Klotho-type co-receptors and heparan sulfat proteoglycans are involved in the initiation of the FGF signaling pathway^[47]. Binding of the growth factor to its receptors leads to autophosphorylation of the receptor molecule which subsequently activates different signal cascades. Activation of the MAP kinase or WNT signaling pathway terminally regulates the transcription programming, whereas PI3K-AKT, Hedgehog, Notch and noncanonical WNT signaling pathway promote the epithelial-mesenchymal transition. Overall, the FGF signaling is involved in numerous biological processes, such as stemness, anti-apoptosis, proliferation, drug resistance, angiogenesis and invasion^[47].

As for many other tumor entities, overexpression of FGF components has been described for gastric cancer, too. The FGFR-2 for instance is known to be up-regulated in 2%-9% of all gastric cancer cases, but is overexpressed in 50% in poorly differentiated and diffuse type gastric cancer^[48].

Currently, there are several experimental studies in progress which evaluate the impact of monoclonal antibodies against FGF-19, FGFR-2 and FGFR-3 at the level of animal models.

Hepatocellular growth factor: Under physiological conditions, Hepatocellular growth factor (HGF) and its corresponding receptor MET play a central role in the embryonic development, wound healing and organ regeneration. Therefore, HGF is normally secreted by surrounding mesenchymal cells^[49,50]. The physiological HGF signal can be altered by numerous molecular disorders, such as gene amplification, mutation and abnormal gene splicing^[51]. Aberrant HGF signaling can be observed in a broad variety of different tumors, among them lung cancer, colorectal cancer, hepatocellular cancer and - as well - gastric cancer. The receptor is activated by receptor dimerization which is induced by binding of HGF. Activation of MAPK and PI3K-AKT signalings are typical subsequent downstream features which lead to cell proliferation, prolonged cell survival and cell mobilisation^[52]. Whereas overexpression of MET seems to be a common feature in gastric cancer (22%-24%), gene amplification is infrequent (2%-10%). Aberrant

HGF signaling is related to poorer overall survival^[53].

Currently, three different monoclonal antibodies targeting the HGF system are available: onartuzumab, rilotumumab and ficlatuzumab^[52].

Onartuzumab has been demonstrated to be beneficial on the level of case reports but did not influence the clinical course in unselected patient populations.

Gastric cancer patients treated with rilotumumab in combination with chemotherapy following the ECX protocol showed a better overall survival as compared with those who received ECX only (5.7% and 4.2%)^[54]. Global phase 3 studies dedicated to the impact of onartuzumab and rilotumumab on advanced gastric cancer are currently underway^[52].

The benefit of ficlatuzumab combined with chemotherapy has been investigated for non-small cell lung cancer but did not have a statistically significant effect on overall survival^[52].

Targeting the growth factor pathways by small molecules

During the last decades two main molecular approaches have been asserted to target growth factor receptors which in fact are complex proteins: Monoclonal antibodies which bind to selected regions on the molecule surface and receptor tyrosine kinase inhibitors (RTKI) which are small molecules. These molecules mimic a metabolite that binds to the active center of the kinase. Two main categories of RTKI can be (more or less) distinguished: RTKIs which bind selectively to one or more related receptor types, and so-called multi-tyrosine kinase receptor inhibitors which have a more pluripotent spectrum of potential receptor targets.

Essentially, RTKI are available for every growth factor receptor. However, clinical outcomes in particular regarding advanced or metastasized gastric cancer show at best moderate improvements in terms of tumor control and survival.

For EGFR gefitinib, erlotinib, lapatinib and dacomitinib have been developed. Gefitinib showed moderate improvement of overall survival in several phase 2 studies. Administration of erlotinib in combination with cytostatic substances led to significant improvement of tumor control in two phase 2 studies. Lapatinib did not show any improvement when administered to patients with advanced, unresectable or metastasized gastric cancer. The benefit of dacomitinib is not clearly evaluated to date^[10].

For VEGFR apatinib is a selective inhibitor. Several studies showed a significant improvement for overall and progression free survival in patients with heavily pre-treated unresectable gastric cancer (OS 6.5 mo vs 4.7 mo, $P = 0.01$)^[12].

Imatinib is a RTKI which targets PDGFR. It is well established in the treatment of gastrointestinal stroma tumors for over 10 years now. A phase 1 study in 2012 showed that imatinib was well tolerated in patients with advanced gastric cancer but did not show significant

clinical improvement regarding survival and tumor control. Dasatinib, a novel PDGFR specific molecule is effective in the treatment of chronic lymphatic leukaemia, the benefit of dasatinib in the treatment of solid tumours is currently investigated^[46].

For the FGFR family a broad variety of small molecules is presented in the literature: dovitinib, brivanib, intendantib and ponatinib to name only a few. However, none of them is established in the treatment of gastric cancer at present^[47].

HGF specific small molecules can be subdivided in three categories: Type 1, 2 and 3.

Type 1 inhibitors are most specific to HGFR, for instance crizotinib. Type 2 inhibitors target a wider spectrum of receptors (AXL, RON, VEGFR2): foretinib, cabozantinib. Type 3 inhibitors bind as well to multiple receptor subtypes and different sites of the respective receptor: tivantinib. For gastric cancer only foretinib reached the level of a phase 2 study but unfortunately without significant benefit on an unselected patient group regarding HGFR expression^[52].

Proteinase-activated receptors

Proteinase-activated receptors (PAR) is a subgroup in the family of G-protein-coupled receptors. Receptor activation is realized by specific serine-proteases, such as trypsin and thrombin, which subsequently leads to further activation of the PI3K signaling pathway. Interestingly, one downstream effect of upregulated PAR2 signaling is the trans-activation of EGF receptors with the known subsequent effects. There is some evidence that prostaglandin-2 may inhibit the PAR2 signaling pathway which could be a potential target for specific molecular treatment approaches, but to date there is no PAR-associated treatment introduced in to the clinical routine^[55].

Morphogens and embryonic signaling pathways

Sonic hedgehog signaling: The Sonic hedgehog signaling (SHH) signaling pathway is one of the key players in the embryonic development, especially in defining body axes and segmental forming. The SHH signal is transduced within the cell *via* patched (PTCH), a transmembranous receptor which subsequently leads to the activation of smoothened and further to the deactivation of a protein complex which normally abolishes Gli, a nuclear factor that can initiate the expression of components of different other pathways, such as WNT, bone morphogenic protein (BMP) and Transforming growth factor β (TGF- β)^[56].

Vismodegib, sonidegib and saredegib are small molecule drugs which inhibit smoothened and thus interrupt the intracellular transmitted SHH signal. Thereby, these molecules mimic the effect of cyclopamine, a naturally occurring SHH inhibitor. The effectiveness of vismodegib in targeted treatment has been described for different tumor entities: With a pilot study on metastatic pancreatic cancer patients it was shown that

vismodegib down-regulates the SHH activity but without statistical significance on survival so far^[57]. Vismodegib has been proven as the very first SHH antagonist for the treatment of basal cell carcinoma in 2013^[58].

Phase 1 studies to verify the clinical eligibility of sonidegib are currently underway. The evaluation of saridegib is at present in the stage of experimental studies.

Another interesting molecular approach towards SHH signaling might be the application of HMG reductase inhibitors, such as statins. The attachment of a cholesterol residue to the SHH molecule is known to be essential to initiate the SHH signaling pathway by SHH. Although to date there are no clinical trials available which introduced statins to clinical use for certain tumor entities, there is some evidence that statins influence the clinical and biological behavior of malignant tumors. Recently, it has been published that statins significantly decrease cancer-specific mortality, particularly in colorectal, prostate and breast cancer.

WNT signaling

WNT signaling is known to be evolutionary highly conserved. During the embryonic development it is mainly involved in cellular differentiation. But also in adults WNT signaling is indeed important, particularly in the stem cell niches of the gastrointestinal tract. Likewise the SHH signaling pathway, the WNT signal starts by binding of WNT ligands to its receptor frizzled which in turn co-acts with LRP and transduces the signal towards the cytosol. To date four different subpathways have been described. In the classical or also called the canonical WNT pathway a multiprotein complex consisting of Axin, GSK3B and APC is being destabilized. This multiprotein complex normally abolishes β -catenin by phosphorylation. The disintegration of the multiprotein complex in turn leads to an accumulation of active non-phosphorylated β -catenin, which subsequently moves to the nucleus and binds to components of transcription (TCF-LEF complex). Interestingly, WNT signaling is coupled to EGFR signaling by at least two mechanisms: First the activation of EGFR signaling leads to internalization of E-cadherin- β -catenin complexes which in turn promotes WNT-dependent gene expression and second E-cadherin inhibits EGFR signaling by preventing receptor dimerization^[59,60].

The following targets have been defined to be eligible to suppress WNT activity: Porcupine (an enzyme that modifies the WNT ligands which is essential for their activity), the frizzled-LRP-dishevelled complex, axin, cyclooxygenase-2, GSK3 β and the TCF- β -catenin complex. Different small molecules targeting porcupine are currently under experimental evaluation, most of them act as competitive ligands to porcupine. They are also called "inhibitors of WNT production"^[61].

Aberrant WNT signaling is frequently observed in gastric cancer. β -catenin is overexpressed in up to 30% of gastric cancer cases, whereas the loss of APC function

occurs in 20% of all gastric cancer cases. SFRP loss, a physiological down-regulation of WNT signaling, is as well frequently to be found in gastric cancer tissue^[62,63].

At the moment there is no WNT associated treatment available for clinical routine, in particular not for gastric cancer.

Notch signaling

As another morphogenic signaling pathway Notch is known to be involved in embryonic organ development as well as in adult stem cell niche regulation. Notch promotes its cellular effects *via* regulation of proliferation, differentiation and apoptosis. The basic molecular mechanism is that one membrane-bound ligand (two subgroups: Jagged 1-2 and Delta like 1-4) binds to its receptor which is membrane-bound, too, but is belonging to a different cell. Thereafter the intracellular component of the receptor is cleaved. The Notch intracellular domain then moves to the nucleus and up-regulates expression of several genes, among them c-myc (oncogene), cyclin D1 (cell cycle promotion), p21 (cell cycle arrest) and bcl-2 (apoptosis)^[64-66].

Notch activity has been described to be involved in several tumor entities and among them in gastric cancer. Particularly Notch 1, Jagged 1 and DLL 4 were found to be frequently dys-regulated in gastric cancer tissues. Furthermore, there were statistically significant differences in the incidence of their up-regulation when stratifying tumor tissues to the classification according to Lauren as well as tumor location and tumor size^[66].

To date, there are no substances available which target at the Notch signaling pathway.

TGF- β and BMP

TGF- β and BMP constitute a super family of morphogens and regulate a broad variety of cellular activities. Up-regulation of the signal cascade may result in antitumor biological effects: At early tumor stages cell differentiation and apoptosis are promoted whereas proliferation is inhibited, leading finally to anti-tumor signals. On the other hand, the up-regulation of TGF- β and BMP in advanced tumor stages may result in the promotion of tumor angiogenesis, cell motility and aberrant interplay with the interstitium^[67-69].

Several subtypes of the TGF- β /BMP family are frequently up-regulated in gastric cancer, for instance BMP7 can be verified in 55% of specimen, whereas BMP2 is up-regulated in almost all cases of gastric cancer and BMP4 up-regulation is a frequently occurring event in undifferentiated gastric cancer.

Dalantercept is an inhibitor of BMP9 and BMP10 which has been shown to suppress effectively tumor angiogenesis. It has been proven to be eligible in a phase 1 study and is now under evaluation as a palliative second line treatment for renal cell carcinoma. DMH-1, a novel small molecule which inhibits the intracellular component of BMP-1 has been shown to have anti-tumor effects in the animal model^[70].

Nuclear factor κ B and interleukin receptors

Nuclear factor κ B (NF- κ B) as well as interleukin signaling are known to be involved in cancer development and cancer progression. NF- κ B can be regarded as a quick time transcription factor that regulates immune reaction as well as proliferation and apoptosis. Extracellular signals like bacterial or viral antigens, interleukin 1 β and tumor necrosis factor initiate a signal which enters the nucleus within few minutes. This is realized by storing NF- κ B in the cytosol which there is inactivated by forming a complex inhibitor of NF- κ B (I κ B). IKK, the I κ B kinase inactivates I κ B, which leads to a NF- κ B release. Rapid movement of NF- κ B to the nucleus in turn leads to up-regulated expression of different genes like cytokines, chemokines and adhesion molecules.

Upregulated NF- κ B signaling in gastric cancer is associated with elevated proliferation, genomic instability and drug resistance.

Two different molecular approaches targeting NF- κ B signaling are at the present time available: Phytochemicals: silibinin (*Silybum marianum*): Prostate cancer; resveratrol (red grapes, red wine): Prostate cancer, mesothelioma; catechins (green tea): Prevention against numerous tumor entities.

The abovementioned agents are partly a domain of alternative medicine but not an integral part of the clinical routine. Systematic studies and randomized trials are needed to shed more light on the actual clinical impact of these treatment options.

Denosumab is an inhibitor of RANKL (receptor activator of NF- κ B) and thus can down-regulate NF- κ B signaling. It has been shown to be effective in giant cell tumor of bone in pre-clinical studies.

To our knowledge currently there is no molecular treatment available targeting the NF- κ B signaling pathway in gastric cancer.

Furthermore, there is an abundance of inflammatory-associated molecular markers which are up-regulated in gastric cancer, including those which are associated with significantly poorer survival, such as different interleukins, HIF-1 α , chemokine receptors as well as matrix metalloproteases (MMP-3, -7, -9, -11).

Components and regulators of cell cycle

Cell cycle up-regulation is one of the most central mechanisms of tumor cell proliferation and tumor growth. It is strictly regulated by different controlling factors. The cell cycle can be sectioned into different cell cycle phases which only can be entered by passing the respective checkpoints. Under physiological conditions the entry of a cell into the cell cycle needs growth factors, whereas in tumor cells the cell cycle can be started at lower levels of growth factors or even at their complete absence^[71,72]. Cyclin D1 and 2 as well as CDK 4 and 6 are the most important factors that promote the entry into the S phase of the cell cycle. Cyclin D1 and 2 are frequently up-regulated in gastric cancer.

Furthermore, cyclin D is an important downstream target of different signaling pathways, such as SHH, WNT and Notch. In 15% of gastric cancer cases an up-regulated cyclin E can be observed^[62,73,74]. The protein complexes formed by cyclin plus its corresponding CDK are inhibited by different factors, such as p21, which is down-regulated in 60% of gastric cancer cases^[75].

Another major cell cycle associated key player is p53, the so-called "guardian of the genome", which is responsible for arresting the cell when DNA is severely damaged. Over 50% of all malignant tumors show a loss of p53, in gastric cancer these are at least 40%. Loss of p53 is known to be particularly frequent in advanced stages of gastric cancer and in those cases when tumor differentiation is low^[76,77].

Cell cycle and its regulators are investigated intensively for several decades to find clinical eligible bonds which inhibit cell cycle activity and promote cycle arrest or apoptosis.

Flavopiridol (also known as alvocidib) as well as roscovitine (also known as seliciclib) can be regarded as CDK inhibitors of the first generation, both of them being relatively unspecific.

After promising results of phase 1 studies with inhibitory effects on multiple different CDK subtypes, the clinical outcomes in phase 2 studies were disappointing failing significant clinical activity. After all, there was a measurable clinical activity in some haematological neoplasms, such as chronic lymphatic leukaemia and mantle cell lymphoma.

Roscovitine, a purine based molecule failed to have clinical effects in as well phase 1 and phase 2 studies^[78,79].

Dinaciclib as a CDK inhibitor of the second generation revealed remarkable activity on numerous tumor cell lines as well as in several tumor mouse models. In the subsequent phase 1 studies dinaciclib resulted in stable disease in different solid tumors, but again the positive results could not be confirmed with phase 2 studies with the exception of palliative treatment in refractory chronic lymphatic leukaemia, so that now a phase 3 study in this field is underway^[78].

The impact of down-regulation of cyclin D1 by using adenoviral vectors is currently explored.

Currently the abovementioned drugs are not approved for clinical use in the treatment of gastric cancer.

SOME FUTURE PERSPECTIVES

Beside the further development of target-specific molecules against components of the abovementioned signaling pathways two categories of molecular tumor biology might be of interest: the clinical importance of micro RNAs and effectors of epigenetic regulation.

MicroRNAs are small molecules without coding function and with a usual length of 18 to 25 nucleotids. To date, more than 2000 different sequences have been detected in the human genome. It is postulated that microRNA molecules are involved in 30% of gene expression. Interestingly they are frequently to be found

at so-called fragile chromosomal sites and typically in intergenic regions. The signature of microRNAs changes from normal tissue to malignant tumor tissue. Micro RNAs can as well be down- and up-regulated.

For example miR-139 has been shown to be frequently down-regulated in gastric cancer. In contrast, overexpression leads to inhibited cell proliferation in gastric cancer cell lines. It seems to be involved in the regulation of the chemokine receptor CXCR4.

The individual signature of microRNAs might be used as a biomarker in predicting the biological behavior of tumors. Furthermore, antagonization of oncogenic microRNAs and the restoration of down-regulated microRNAs with tumorsuppressive activity might be promising targets in the future^[80].

To a certain degree, the function of microRNA molecules is associated to epigenetic mechanisms, another challenging future perspective towards better understanding of the molecular biology of gastric cancer. Epigenetics means methylation of the DNA strand as well as different modifications of the histone molecules. DNA methylation is realized by DNMT 1 and 2 which place the methyl residues predominantly at so-called CpG rich regions. Hypermethylation of promoter regions upstream of tumor suppressor genes is a commonly observed phenomenon in different solid tumors. Histone molecules can be acetylated by HAT and deacetylated by HDACs at lysine sites, furthermore lysine as well as arginine sites can be methylated or demethylated. A broad variety of dys-regulated histone modification has been described for gastric cancer, for instance the hyperacetylation of histones neighboring the myc oncogene. Restoration of dysregulated histone and DNA modification might be another promising target to anticancer treatment^[81].

Considering the variety of target specific therapeutics in relation to the clinical impact on the population of gastric cancer patients and the individual complexity of the "cancer organism" it becomes clear, that molecular targeted approaches generate their best effects on respective subgroups which harbour the suitable molecular signature. Therefore, the knowledge about the individual presence of molecular markers might become essential and of paramount interest in the future.

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P- Reviewer: Bologna M

S- Editor: Yu J **L- Editor:** A **E- Editor:** Jiao XK





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