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Molecular Features and Their Clinical Implications on Gastric Adenocarcinoma

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1. Introduction

Gastric cancer is one of the most frequent malignant tumors worldwide. It is known to have a poor prognosis leading to more than 600.000 fatalities every year. This dilemma is caused mainly by the late onset of clinical symptoms. Thus, when gastric cancer is diagnosed, more than 50% of patients cannot be cured.

Even in those cases where cure by surgery can be achieved, the prognosis is poor due to the high incidence of early recurrence of the primary tumor as well as the development of metachronous metastases. The attempt to improve survival by extended surgical procedures, such as extensive lymph node removal did not have a significant impact on survival leading medical professionals to regard advanced gastric cancer as a systemic rather than a locally confined disease. Therefore, over the last two decades the importance of chemotherapy as a part of the curative treatment strategy was became more evident. The findings of molecular research show that gastric cancer has a very heterogenous biology with a broad variety of signalling pathways and chromosomal imbalances involved. With the growing insight into the molecular biology of gastric cancer it became clear that every single gastric cancer has to be regarded as an individual entity which can change its molecular behavior over time. This chapter presents the current knowledge of the molecular profile of gastric cancer. The most important basic signalling pathways that are known to play critical roles in the development as well as the progression of gastric cancer are discussed. Furthermore, the currently available molecular targets and their clinical relevance are reviewed in this chapter.

2. Chromosomal imbalances in gastric cancer

To date, the TNM system and the classification according to Lauren are the "gold standard" tools to predict survival and to define the treatment strategy in patients with gastric cancer. However, there is a wide range of clinical outcomes in patients with similar TNM stages. Therefore, it seems to be important to improve the characterization of gastric cancer by using molecular criteria [1].

The development as well as the progression of gastric cancer is the result based on the interplay between the host genetic profile and the environment. The precise transfer of genetic information requires accurate replication, DNA mismatch repair and the segregation of replicated DNA strands. Whereas an impaired replication is not compatible with cellular

survival, the uneven distribution of chromosomes in tumor cells is termed aneuploidia and can be frequently observed in gastric cancer tissue. Defects in the mismatch repair system can gradually lead to chromosomal imbalances. Functional losses of MLH1 and MSH2 are known to cause multiple somatic mutations which may present microsatellite instability and may lead to tumor formation at multiple locations at a younger age in the colon /rectum (Lynch syndrome). Most frequently, the hypermethylation of CpG islands in the promoter region of these mismatch repair genes is the underlying mechanism of loss of function. This type of tumor development is called microsatellite mutator phenotype and has also been described in gastric cancer by several authors [2,3]. The other type of tumor development based on chromosomal imbalances is the chromosomal/ intrachromosomal instability type leading to amplifications and deletions of whole chromosomes as well as chromosome cytobands potentially harbouring tumor suppressor genes or oncogenes. The following locations have been observed to be frequently amplified in gastric cancer: 3p22, 4q25, 8q24, 11p13, and 20q13. Losses have been documented most commonly at 1p36 and 9p21 [4]. Furthermore, it has been reported that intestinal type gastric cancer according to Lauren´s classification has a higher degree of chromosomal aberrations than the diffuse type. Furthermore, a relationship between clinical outcome and the degree of chromosomal aberrations in intestinal type gastric cancer was observed [5].

Another level of chromosomal assessment is the detection of gross chromosomal imbalances by using flow cytometry. The loss or amplification of complete chromosomes is known to be a frequently occurring event in cancer development [6]. Non-diploid status of chromosomes has been shown to be present in about 20% of gastric cancer cases. Patients with a non-diploid chromosome status have a significantly poorer survival than those who present with a diploid status [1].

Additionally, there is a growing body of evidence that the epigenetic level of regulation of gene expression plays an important role in cancer development. The chromatine remodelling machinery contains in particular enzymes that modify histons [7]. Recently, it has been shown that the histone demethylase RBP2 is upregulated in gastric cancer and that depletion of that enzyme causes senescence of resident tumor cells [8].

3. Hereditary gastric cancer

In 10% of all gastric cancer cases, a familial clustering of the disease can be observed but only 1 -3% are of hereditary origin. In those cases where no histological finding is available the term “familial gastric cancer” is used [9]. The following syndromes include patients with available histology and are known to be associated with both familial clustering and certain germline mutations: hereditary diffuse gastric cancer, familial diffuse gastric cancer and familial intestinal gastric cancer. Furthermore, there are several syndromes which may include early onset gastric cancer in addition to other tumor manifestations: Lynch syndrome, Li-Fraumeni syndrome, Li-Fraumeni-like syndrome. Peutz-Jeghers syndrome and the familial adenomatous polyposis (FAP).

Hereditary diffuse gastric cancer develops from germline mutations in the *CDH-1* gene (about one third of cases) coding for E-cadherin, an important cell adhesion molecule on the lateral surface of the cellular membrane, whereas in two thirds of the cases the underlying mutation cannot be identified [9,10]. The inactivation of the *CDH-1* gene may be triggered by epigenetic mechanisms (hypermethylation of the promoter region) as well as by a genetic mechanism (loss of heterocygosity plus *CDH-1* mutation). The syndrome is associated with

early onset and gastric cancer lesions growing in multiple locations as well as with lobular breast carcinoma. The International Gastric Cancer Linkage Consortium defined two main criteria to identify patients susceptible for hereditary diffuse gastric cancer from the population: 2 cases of diffuse gastric cancer in first or second degree relatives (at least one of them to be younger than 50 years at the time of diagnosis) or 3 cases of diffuse gastric cancer in first or second degree relatives independently of the age at the time of diagnosis. Families with numerous cases of diffuse gastric cancer who do not fulfil these abovementioned criteria are considered to be familial diffuse gastric cancer [11].

The majority of cases of familial intestinal gastric cancer is associated with Lynch syndrome which is also termed hereditary non-polyposis colorectal cancer (HNPCC) [12,13]. Therefore, Caldas and co-workers suggested to identify patients with risk for familial intestinal gastric cancer by using the HNPCC-related Amsterdam criteria in countries with a high incidence of gastric cancer. In contrast, countries with a lower incidence should use the criteria analogous to hereditary diffuse gastric cancer [13].

Lynch syndrome is caused by germline mutations in genes associated to the mismatch repair (MMR) machinery. Most frequently, alterations of the *MLH* gene family (MutL-homolog) or the *MSH* gene family (MutS-homolog) are responsible for the defective MMR. These two proteins form heterodimers that recognize mismatched bases selectively within the newly synthesized daughter DNA strand which can be removed subsequently by exonucleases. Appoximately 5 – 8% (depending on gender and the mutated gene) of patients with Lynch syndrome develop gastric cancer during their life time [12,14].

The Li-Fraumeni syndrome as well as the Li-Fraumeni-like syndrome originate from germline mutations of the *TP53* gene. About 250 different mutations of that gene have been identified to cause a broad variety of tumor diseases, the majority of them being missense mutations leading to a molecule that cannot bind to the DNA [15]. There is a broad variety of tumor diseases which can develop based on that syndrome including breast cancer, adrenal tumors, sarcomas and gastric cancer. The Li-Fraumeni syndrome and the Li-Fraumeni-like syndrome are distinguished by several clinical based criteria. Recently it has been demonstrated that the DNA repair gene *BRCA2* might be another molecular mechanism of causing these syndromes [16].

Peutz-Jeghers syndrome (*STK11* gene, coding for serin-threonin kinase 11) and the FAP (associated to the *APC* gene) can are associated with gastric cancer in rare cases.

4. Dysregulation of signalling pathways

Non-hereditary gastric cancer has a complex and multistep carcinogenesis in which – among numerous other factors – signalling pathways are involved. The dysregulation of these signalling pathways may lead to activation of genes that code for proteins regulating growth processes, such as cell division, differentiation, epithelial-mesenchymal transition and proliferation. Several studies on the molecular development as well as the progression of gastric cancer show clearly that there are differences in the biological behavior between young and elderly people. According to the classification of Lauren, intestinal gastric cancer with differentiated glandular growth pattern presents with a profile of dysregulated signalling pathways that can be distinguished from the diffuse type gastric cancer. Furthermore, there appears to be a difference between cancer of the upper stomach as compared to that of the lower stomach.

4.1 Tyrosine kinase signalling

4.1.1 The epidermal growth factor receptor (EGFR) family and downstream signalling

Epidermal growth factor receptor is a member of the ErbB receptor family and normally regulates gastric mucosa proliferation. Under normal conditions, two receptor molecules form dimers as a result of ligand binding and phosphorylate each other via their tyrosin kinase activity. Every heterodimer has its own effector molecule (see Fig. 1). One important pathway is the activation of the guanine exchange factor SOS which subsequently activates the MEK-ERK MAP kinase pathway leading to the expression of proliferation-promoting transcription factors, such as AP-1, ELK-1 and c-fos. Alternative signal transduction pathways downstream of the EGF receptor family are STAT and PKB (protein kinase B). An important downstream effector of PKB is the mTOR complex 1. Active mTOR complex 1 leads via expression of specific mRNA stabilizing proteins and of ribosomal subunit S6 to increased proliferation.

In summary, EGFR related signals promote many cellular activities towards tumor growth, in particular proliferation, migration, adhesion, apoptosis and differentiation [17].

EGFR	7p11.2	EGFR	EGF TGFα
ERBB2	17q12	HER2/NEU	none
ERBB3	12q13.2	HER3	neuregulin1 neuregulin2
ERBB4	2q34	HER4	neuregulin1 neuregulin2

Fig. 1. The members of the EGF receptor family and their typical ligands

Dysregulation of signalling pathways downstream of the EGFR is frequently observed in gastric cancer. Therefore, currently two main subgroups of targeted drugs exist: first the inhibition of the receptor by blocking the ligand binding site and second the inhibition of the intracellular tyrosin kinase activity of the receptor molecule. For the first-mentioned mechanism the monoclonal antibodies cetuximab, matuzumab and panitumumab have been developed. Gefitinib and erlotinib are tyrosin kinase inhibitors.

The ErbB2 receptor (Her2/Neu) can dimerize without a binding ligand and, thus, cannot be inhibited by antibodies against the ligand binding site. About one third of all tumors express ErbB2. In up to 27% of gastric cancer cases an overexpressed ErBB2 can be detected [18]. ErbB2 is overexpressed more frequently in intestinal type gastric cancers as compared to diffuse type gastric cancer. Furthermore, younger patients show less frequent ErbB2 overexpression than elderly patients [19]. The humanized antibody trastuzumab inhibits the ErbB2 receptor and has been shown to provide a significant survival benefit in ErbB2-positive cases [20].

Recently, it has been shown in vivo and in vitro on gastric cancer cell lines that the mTOR specific inhibitor rapamycin is effective in those cases which are resistant to EGFR inhibitors. Moreover, when administered in combination with cetuximab it can restore the anticancer effects of EGFR inhibition. Thus, rapamycin alone or in combination with other agents may provide a new target for the clinical use in the future [21].
K-ras, BRAF and SOS mutations are detected only in few gastric cancer cases [22]. Nevertheless the novel k-ras inhibitor tipifernib should be mentioned. K-ras mutations result in a continuosly active k-ras protein. Tipifernib inhibits the enzyme farnesyltransferase which forms an essential step in the posttranscriptional modification and thereby downregulates the k-ras signal. The clinical outcome of tipifernib administration in advanced gastric cancer is currently investigated in a phase 2 trial and might be a potential target-specific therapy for selected gastric cancer cases in the future, too [23].

4.1.2 Vascular endothelial growth factor (VEGF) and tumor angiogenesis

When tumors grow larger than 1-2mm in diameter, further tumor growth requires the development of new blood vessels in the tumor environment. This tumor-related angiogenesis is different from physiological angiogenesis in terms of distinct vessel architecture, vascular permeability as well as a different interplay between endothelial cells and perivascular cells. One of the most potent angiogenesis-promoting factors is VEGF. VEGF is expressed as a result of tissue hypoxia. It promotes its biological effects (inhibition of apoptosis and proliferation of endothelial cells, increased endothelial cell migration) via binding to the VEGF receptor. Synchronously with the once initiated tumor angiogenesis the development of metastases is promoted.
Overexpression of VEGF has been reported in gastric cancer cases.
Currently, 4 different mechanisms of VEGF signalling inhibition can be distinguished: VEGF-inhibiting antibodies (bevacizumab), soluble VEGF receptors (aflibercept), VEGFR-inhibiting antibodies (IMC-1121B) and VEGFR tyrosin kinase inhibitors (vatalinib, cediranib, sunitinib, sorafenib).
To date, for gastric cancer no angiogenesis-inhibiting agent has been approved worldwide. But a growing experience with angiogenesis-inhibiting agents in colorectal cancer demonstrates a survival benefit with acceptable adverse effects [24].

4.1.3 Hepatocellular growth factor (HGF) and gastric cancer

HGF signals induce a broad variety of biological features, such as morphogenesis, adhesion, migration, remodelling of extracellular matrix and are also involved in tumor angiogenesis [25]. It is known to be one of the key players in tissue regeneration following injury. Under physiological conditions, HGF is secreted in a paracrine manner most commonly by mesenchymal cells. Its target, the HGF receptor (HGFR or MET) is usually present on the surface of epithelial cells. Several cytokines, such as interleukin-1 and -6, tumour necrosis factor-α and transforming growth factor-β (TGFβ) that are released into the reactive interstitium (wound healing, cancer) induce the upregulation of HGF and HGFR expression. Furthermore, HGF is a strong antagonist of liver fibrosis by inhibiting the TGFβ signal as well as by pro-apoptotic effects on myofibroblasts. [26]. Intracellular signal transduction is similar to the EGFR signalling pathways.
In gastric cancer, the upregulation of the HGF signalling is an event which predominantly occurs at advanced stages, in particular in those who have liver metastases [27]. About 23% of patients with gastric cancer have an overexpression of c-met [28].

There are currently no substances available for clinical use that inhibit the HGF signalling pathway but the effect of the novel agent XL880 (a tyrosin kinase inhibitor) on poorly differentiated gastric cancer is currently investigated. Furthermore, antibodies against HGF and MET are planned [29].

4.1.4 Fibroblast growth factor and its receptor FGFR in gastric cancer

Like EGFR and HGFR, the fibroblast growth factor receptor belongs to the large family of receptor tyrosin kinases. To date, 23 different isoforms (FGF 1-23) and 4 receptor subtypes (FGFR 1-4) have been identified. FGFR2 or k-sam has been reported to be overexpressed in poorly differentiated gastric cancer The MAP-kinase pathway, the AKT pathway as well as NFAT signalling and activation of protein kinase C are the most prominent subsequent intracellular transduction pathways. Recently it has been shown that the receptor of keratinocyte growth factor on epithelial cells is identical with FGFR2. The amplification of the k-sam gene has been detected in poorly differentiated diffuse gastric cancer in about 50% of cases resulting in a poor prognosis [30,31]. In contrast, it is rarely present in intestinal type gastric cancer.

The novel FGFR2 inhibitor Ki23057 showed considerable antiproliferative effects in the mouse model and might become an effective target-specific agent in the future.

4.1.5 Vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF) and its receptor PDGFR in gastric cancer

VEGF and PDGF are key players of angiogenesis. Whereas VEGF promotes neoangiogenesis, PDGF has its impacts on the maintainance of microvessels. Both factors are secreted by tumor cells and act on the surrounding tumor microenvironment. There are protein families for both factors, the most important subtypes in gastric cancer are VEGF-A and PDGF-B. The signals are mediated via binding to VEGF- and PDGF receptors which have an autophosphorylating tyrosin kinase activity and subsequently initiate intracellular signal cascades that result in migration, proliferation and angiogenesis.

The overexpression of both signal molecules has been shown to be correlating to each other in gastric cancer. PDGF-B seems to play a more important role in intestinal type gastric cancer whereas VEGF-A is more important for angiogenesis in diffuse type gastric cancer [32].

The PDGF receptor can be inhibited by imatinib. Imatinib is a specific tyrosin kinase inhibitor that is frequently used in gastrointestinal stroma tumors. A potential treatment option in the future might be the novel multiple receptor tyrosine kinase inhibitor LY2457546 which has been shown to have anti-tumor effects in animal models recently.[33]

4.2 Proteinase-activated receptors (PAR) in gastric cancer

Proteinase-activated receptors belong to the family of G-protein coupled receptors. The receptor is activated by serin-proteases, such as thrypsin and thrombin by removing an N-terminal segment on the extracellular space. The thus created neo-N-terminal end subsequently serves as an intramolecular ligand that binds to the binding domaine. The signal is then transferred to the intracellular space leading to activation of several signal transduction pathways. In gastric cancer cell lines, PAR2 has been shown to trans-activate the EGF receptor. This effect could not be blocked by EGFR inhibitors [34]. To date, there are no inhibitors of PAR evaluated for clinical use.

4.3 WNT signalling and the involvement of E-caherin

The WNT signalling pathway belongs to the most important signalling systems and is evolutionary highly conserved. It plays a central role in embryonic differentiation processes. In adults, WNT activity can be detected within the stem cell niche of the gastrointestinal tract. Furthermore, there is a growing body of evidence that the WNT signalling pathway is a key player in the progression of tumors. The signal originates in the extracellular space with the binding of WNT (several subtypes exist) to its receptor frizzled. In co-operation with the co-receptor LRP the signal is then transduced to the intracellular space. From here, 4 different signalling cascades can be activated, which is shown in more detail in Tab. 1. In the classical or canonical WNT pathway the WNT signal leads to the destabilization of the multiprotein complex consisting of Axin, GSK3B and APC. GSK3b normally phosphorylizes beta catenin which in turn leads to the degradation of beta catenin. Intact beta catenin moves to the nucleus and binds to TCF/ LEF complex which constitutes a transcription factor with the subsequent expression of specific genes.

WNT sub-pathway	Targets	Biological effects
Canonical WNT/ beta catenin pathway	Cyclin D1, c-myc, MMP-7, FGF 20, DKK 1	Embryonic development, differentiation, tumor progression
WNT/JNK Pathway = planar cell polarity pathway (PCP)	Cytosceleton	Cytosceletal remodelling, cell polarity
WNT/ calcium pathway	PKC, PLC	Cell adhesion
Asymmetric cell division pathway	Mitotic spindle	Asymmetric cell division

Table 2. Several pathways downstream to the WNT signal

It is of importance to know that the WNT signalling pathway is part of a complex network of signal transduction which is controlled and regulated by a multitude of factors belonging to different pathways. In particular, a complex crosstalk between WNT, E-cadherin and beta-catenin has been described. E-cadherin inhibits dimerisation and activation of the EGFR via binding to EGFR monomers. On the other hand, activation of EGFR leads to the internalization of E-cadherin-beta-catenin complexes which in turn induces expression of WNT dependent target genes [35].

About 30% of gastric cancer cases show an overexpression of beta-catenin which is associated with poorer prognosis than beta-catenin-negative gastric cancer. The loss of APC function which is essential for the beta-catenin degradation is observed in about 20% of gastric cancer cases. The downregulation of SFRP – an inhibitor of the WNT receptor – by methylation of the promoter region has been described to occur in gastric cancer, too [17,36].

To date, there are no WNT specific anticancer drugs available. Recently, it has been demonstrated that differentiation-inducing factors downregulate the WNT pathway by activating the GSK3B in several cancer cell lines [37].

4.4 Sonic hedgehog signalling pathway (SHH)

The basic structure of SHH shows some similarity with the WNT pathway: An extracellular ligand (SHH) binds to a receptor at the cell surface (patched) which transduces the signal into the cell. Thereby, an intracellular signal transducer (smoothened) is activated which in turn deactivates a multiprotein complex (fused, suppressor of fused, coastal and others).

This deactivation leads to accumulation of a nuclear factor (Gli) which is normally degraded by the functional multiprotein complex. Eventually, specific genes encoding proteins, such as cyclin D2, patched and Gli (positive feedback) are activated. There is a ample crosstalk with other signalling pathways, in particular the expression of TGF-beta (TGF-beta pathway), the expression of SFRP1 (WNT pathway) and FOXL1 (bone morphogenic pathway).
The sonic hedgehog signalling plays a central role in embryonic development and has been shown to be upregulated in a broad variety of malignant tumors [38]. The overexpression of both Gli and the receptor patched have been reported in two thirds of gastric cancer cases. It has been shown that SHH expression is stronger in intestinal type gastric cancer and tubular growth pattern than in diffuse type gastric cancer and mucinous or undifferentiated growth pattern [39]. SHH is also overexpressed in non-malignant lesions, such as gastric adenomas or intestinal metasplasia. Due to the upregulation of SHH signalling at an early stage of cancer development it might serve as an early diagnostic tool in patients who have an increased risk for the development of gastric cancer.
The SHH signalling pathway theoretically could be blocked by cyclopamine. This substance was first discovered on sheep that fed on a certain lilieaceous plant (veratrum californicum) and delivered lambs with only one eye, reflecting the crucial role of SHH in the differentiation of embryonic body axes. Currently the design of a fully synthetic cyclopamine analogon is underway. Another potential inhibitor of SHH signal might be HMG reductase inhibitors, such as statins. HMG reductase inhibitors are necessary to process the functional SHH molecule. To date, there are no studies available on the effect of statin-dependent downregulation of SHH signals by statins and its effects on clinical outcome in the field of gastric cancer.

4.5 Notch signalling pathway and gastric cancer

Notch signalling belongs to the morphogenic embryonic signalling pathways. Notch is involved in the regulation of cellular proliferation, apoptosis and differentiation. It participates in organ development and mediates lateral inhibition of neighboring cells. The receptor (notch) and its activating ligand (delta) are located on the cell surface of two different cells. The binding of delta to its receptor notch leads to a two-step cleavage of the receptor. The intracellular receptor residue then moves to the nucleus where it forms a complex with other proteins and in turn promotes specific gene expression. Notch-specific target genes encode for several members of the Hes-family (regulation of embryonic neurodermal development), CD 25 (interleukin receptor 2, parts of the T-cell receptor), GATA3 (transcription factor for T-cell maturation), c-myc, cyclin D1, p21 bcl-2 [40-46].
Upregulated notch signalling by the ligand Jagged1 has been reported to occur frequently in gastric cancer. This upregulation was associated with poorer survival. To date, there is no target-specific treatment introduced in the current literature.

4.6 The transforming growth factor beta (TGFbeta)/ bone morphogenic protein (BMP) family and signalling pathways in gastric cancer

TGFbeta-signalling is another member of the family of embryonic developmental pathways. In cancers, TGFbeta signalling has a bivalent function: at an early tumor stage it serves as a tumor suppressor by inhibiting proliferation and promoting cellular differentiation as well as apoptosis. These processes are regulated via theRunx3 and the SMAD4 protein [17].

During cancer progression, the corresponding genes are silenced by methylation or otherwise undergo loss of function. The tumor suppressive function changes dramatically in advanced tumor stages where BMP promotes tumor angiogenesis, cell motility and the interaction of tumor cells with the interstitium. Furthermore, the immune response is suppressed by BMP [47,48]. The tumor promoting function is realized via several MAP kinase signalling pathways.

Similarly, BMP signals have bivalent functions: whereas BMP is upregulated in gastric inflammation it is downregulated in gastric cancer. On the other hand, BMP2 has been shown to promote tumor cell motility and, thus, invasiveness of gastric cancer. Furthermore, activins that constitute another subgroup of the TGFbeta/ BMP superfamily have tumor suppressive effects via activation of several caspases (pro-apoptotic), activation of p21 (cell cycle arrest) and downregulation of bcl-2 (anti-apoptotic) [49]. In summary, TGFbeta, BMP and activin signals can be regarded as a tumor suppressive mechanism restricted to certain stages of tumor progression [17].

In scirrhous gastric cancer cell lines it has been observed that treatment with two novel TGFbeta receptor 1 inhibitors (Ki26894 and A-77) decreases biological tumor progression features, such as invasiveness and epithelial-mesenchymal transition. These compounds are potential targeted drugs for metastatic scirrhous gastric cancer in the future [17,50,51].

4.7 Cell cycle dysregulation in gastric cancer

The cell cycle is strictly regulated by a broad variety of controlling factors. To move the cell cycle machinery forward, several checkpoints that control the entry to the next cell cycle phase have to be passed [52]. The most important checkpoint is the G1/S-checkpoint which regulates the initiation of cell division [53]. Under physiological conditions growth factors and other signals are needed to open the gate but in cancer cells the cell cycle can be active without the presence of activating signals and furthermore might be accelerated by overexpressed growth factors as mentioned above. Figure 2 illustrates the different checkpoints as an overview.

Both cyclin D and E in combination with the corresponding cyclin dependent kinase CDK 4/6 and CDK2 are essential for the S phase entry and the subsequent activation of the retinoblastoma protein [54]. Both cyclin D1 and 2 are known to be frequently upregulated in gastric cancer [17,54]. Cyclin D subtypes are downstream targets of several signalling pathways (Notch, SHH, WNT) whereas cyclin E has been reported to be upregulated by gene amplification in about 15% of gastric cancer cases [55].

The *TP-53* tumor suppressor gene is due to its various functions also called the "guardian of the genome". The protein p53 recognizes signals that point to DNA damage, initiates cell cycle arrest for damage repair (by activating target genes that encode for p21), functions as a transcription factor that activates expression of DNA repair genes and also can promote cell death via apoptosis in cases of irreparable DNA damage [56]. About 50% of all tumors and 40% of gastric cancer cases present with *TP-53* mutations resulting in a loss of p53 function. Loss of p53 is frequently associated with advanced tumor stages and undifferentiated tumors reflecting the increased accumulation of mutations within the tumor cell genome [57].

The loss of function of the tumor suppressor p21 occurs in 60% of gastric cancer cases and is associated with increased invasiveness, metastasis and poor prognosis. Moreover, the

incidence of that loss increases with advancing tumor stages. The p27 which is an inhibitor of the CDK4-cyclinD complex and the CDK2-cyclinE complex as well arrest the cell cycle at the restriction checkpoint [58]. The loss of p27 similarly to p21, occurs in gastric cancer in dependence of tumor stage and predicts poor prognosis [59].

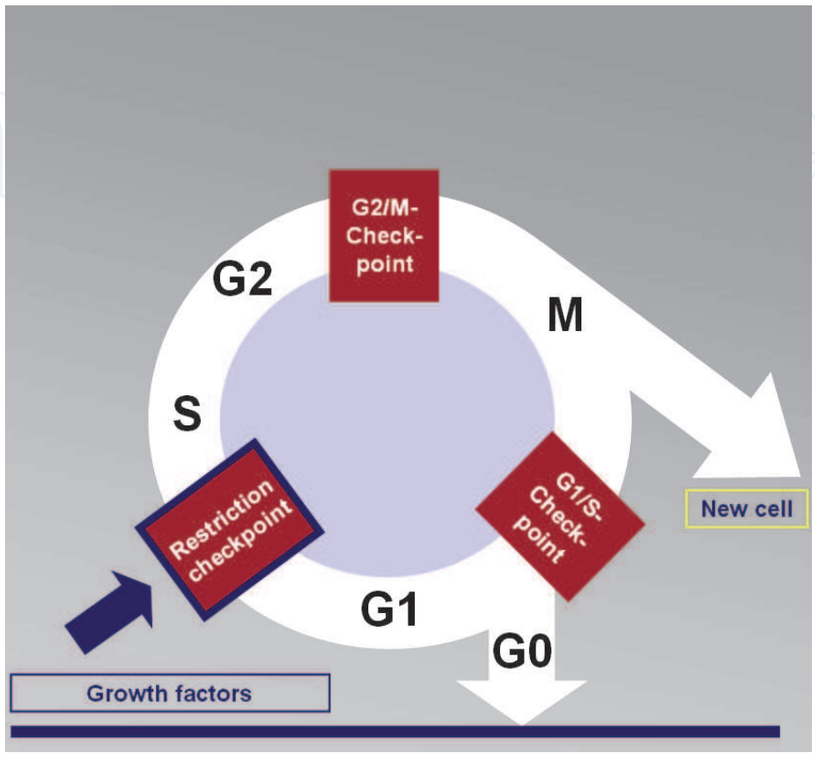

Fig. 2. Checkpoints of cell cycle

4.8 Nuclear factor kappa B in gastric cancer

Nuclear factor kappa B (NFkappaB) is a quick-time transcription factor that modulates the immune reaction and regulates proliferation and apoptosis. Extracellular signals (interleukin 1beta, tumor necrosis factor alpha via their receptors) as well as free radicals, bacterial and viral antigens are transmitted to the nucleus within minutes. This is accomplished by storing NFkappaB bound to a complex (IkappaB) in the cytoplasma from which NFkappa B is mobilized via inactivation of IkappaB by a IkappaB specific kinase (IKK). The free NFkappaB molecule can then move within the nucleus and activate specific genes like cytokines (IL-6, IL-1beta, TNF), chemokines (IL-8, CCL2, CCL3), enzymes (iNOS, COX-2) and adhesion molecules (VCAM-1, ICAM-1, CEACAM-1) which in turn promote inflammatory processes [60].

NFkappaB plays a central role in inflammatory cancers. Dysregulation of the NFkappaB has been observed in gastric cancer to be associated with increased proliferation, genomic instability and drug resistance [61,62].

There is a variety of phytochemicals, such as silibinin, resveratrol and catechins which are known to suppress the activation of NFkappaB. Also, several poly-phenol bonds (green tea) can act as anti-cancer drugs. Furthermore, inhibitors of IKK are studied at a preclinical stage [60].

4.9 S-100 proteins in gastric cancer

The S-100 protein family currently counts 21 members which are responsible for various cellular functions, in particular they are assumed to be second messenger molecules which interact with numerous different molecules. S-100A2, 3, 4, 7 and 10 have been observed to be overexpressed in gastric cancer.

S-100A4 is regulated by epigenetic mechanisms and by beta-catenin. Overexpressed S-100A4 was associated with advanced tumor stages, diffuse type gastric cancer and increased incidence of tumor lymph node involvement [63]. S-100A4 has been demonstrated to be upregulated predominantly in early stages of gastric cancer [64], other studies associated this protein to metastasis in several tumors and, therefore, it is also named metastasin. S-100A4 has a multitude of binding partners, such as E-cadherin, p53, actin and p37 among many others. Interestingly, S-100A4 is one of the strongest molecular based predictors of survival in patients with ovarian carcinoma, pancreatic cancer and gastric cancer [65].

Moreover, calcyclin-binding protein has been shown to inhibit proliferation and invasiveness in gastric cancer by promoting S-100-protein ubiquitinylation which might be a potential target-specific treatment option in the future [66].

4.10 Dysregulated DNA repair mechanisms

The accurate transfer of genetic information through the DNA replication and cell division machinery is dependent on both, exact DNA synthesis procedure and sufficient DNA damage repair systems. Moreover, the DNA is permanently exposed to toxic metabolites, such as oxygen radicals and to physical stress, such as ultraviolet radiation. In addition, spontaneous mutations and DNA methylation occur. In particular, 4 basic mechanisms of DNA repair exist: 1.) the single base excision system, 2.) mismatch repair system, 3.) the nucleotide excision repair system and 4.) the double strand break repair system.

The single base excision system removes defective and modified bases. Therefore, two mechanisms exist: either the aberrant base is recognized and selectively removed by specific DNA glycosylases resulting in an apurinic or apyrimidic ribosyl residue which is in turn cleaved by a AP endonuclease; or the base is repaired directly without removing.

The mismatch repair system recognizes mismatched single bases, small single strand loops and the recombination of non-homologous sequences. Heterodimers from MLH2, 3 and 6 recognize and bind to those DNA loci which in turn leads to the recruitment of further proteins (helicase, single strand binding protein, MSH, and others) forming a protein complex. Hereditary defects in this system are known as as the Lynch syndrome.

The nucleotide excision repair system in particular is responsible for recognizing and repairing DNA damage that is caused by solar radiation. As a logical consequence those patients who have genetic defects concerning this system primarily present with dermal diseases due to the direct exposition of the skin to solar radiation (e.g. xeroderma pigmentosum). It is the only one repair system that is able to remove bulky adducts (dimers of pyrimidin and cyclobutan) and remove up to 32 nucleotides. More than 30 genes are involved in that repair mechanism system.

The double strand break repair system can either act with homologous recombination (HR) immediately behind the replication fork or can act with non-homologous endjoining (NHEJ). The first mechanism requires a sister chromatide which is not available during G0 and G1 phase, therefore in these phases of cell cycle only NHEJ can be performed. The frequently discussed tumor suppessor proteins BRCA1 and 2 are involved in the HR repair system [67,68].

Defective DNA repair systems cause the accumulation of mutations which can lead to transformation provided that the mutation provides a selection benefit. Therefore, it is not surprising that genetic defects in DNA repair are associated with the development of malignant tumors, as it is the case in about 15% of all colorectal cancer cases as well as in other tumor entities like ovarian carcinoma and endometrial carcinoma. For gastric cancer it has been reported that about 8% of sporadic cases show microsatellite instability pointing to defective DNA mismatch repair [69].
PARP inhibitors which block the enzyme poly(ADP-ribose) polymerase might a become potential target specific therapy in cases of mutated BRCA1 and 2. The substance psoralen that induces interstrand crosslinks is investigated in preclinical studies [67].

5. Conclusion

A growing body of knowledge about the biological behavior as well as the molecular nature of gastric cancer is available in the current literature. Although there are only few targeted treatment strategies implemented in the clinical routine a large number of studies dedicated to molecular based strategies is currently underway.
This chapter also implicates that there is a complex pattern of molecular mechanisms that extend the interpretation of gastric cancer in addition to classifications that are exclusively based on macroscopic and histological findings. The pattern of genetic imbalances and of dysregulated pathways is of essential importance to treat patients in a more individualized fashion.

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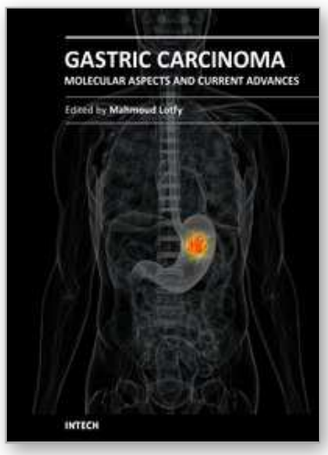

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Gastric cancer is one of the most common tumors worldwide. It has a heterogeneous milieu, where the genetic background, tumor immunology, oxidative stress, and microbial infections are key players in the multiple stages of tumorigenesis. These diverse factors are linked to the prognosis of the gastric cancer and the survival of gastric cancer patients. This book is appropriate for scientists and students in the field of oncology, gastroenterology, molecular biology, immunology, cell biology, biology, biochemistry, and pathology. This authoritative text carefully explains the fundamentals, providing a general overview of the principles followed by more detailed explanations of these recent topics efficiently. The topics presented herein contain the most recent knowledge in gastric cancer concerning the oncogenic signaling, genetic instability, the epigenetic aspect, molecular features and their clinical implications, miRNAs, integrin and E-cadherin, carbohydrate-associated-transferases, free radicals, immune cell responses, mucins, Helicobacter-pylori, neoadjuvant and adjuvant therapy, prophylactic strategy for peritoneal recurrence, and hepatic metastasis.

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