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Short Review

Neoadjuvant Chemotherapy in Barrett's Carcinoma – Prognosis and Response Prediction

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Abstract. *In spite of endoscopic surveillance programs, 90% of patients initially presenting with Barrett's carcinoma have locally advanced disease. In these patients, preoperative chemotherapy increases the chance of a curative resection in responding patients. Unfortunately, response occurs in only 50% of patients after chemotherapy with cisplatin, 5-fluorouracil and leucovorin. Response prediction seems to be possible by measuring metabolic activity by positron emission tomography (PET) scan. Differentiation of responders from non-responders even before starting chemotherapy might be possible using microarray technology and immunhistology in tumour biopsies. A pattern of at least two-fold differentially regulated genes comparing responding and non-responding oesophageal adenocarcinomas was identified. The strongest difference can be seen for tumour necrosis factor, polyribonucleotide nucleotidyltransferase and the ephrin-B3-receptor. In conclusion, our experience suggests that it may be possible to characterize patients responding to chemotherapy by PET two weeks after starting the chemotherapy or even before treatment using customized microarray analysis.*

Barrett's Carcinoma

The incidence of oesophageal adenocarcinoma has been increasing at an alarming rate in Western world during the last 20 years (1-3). Patients with chronic gastroesophageal reflux have a 30- to 40-fold increased risk for adenocarcinoma of the oesophagus, which increases with Barrett's metaplasia to 100-fold (1, 3-5). Early diagnosis is only possible with endoscopic surveillance programs. In spite of these programs, 90% of patients present with at least a locally advanced or even metastatic tumour and fewer than 10% have early-stage cancer (5-8). The sequence from normal squamous cell epithelium to an intestinal metaplasia to dysplasia and finally invasive carcinoma is associated with several risk factors, such as an acidic or biliary reflux, the composition and the duration of the reflux and alimentary carcinogens (9-11).

Reid *et al*. showed that patients with no or low-grade dysplasia develop Barrett's carcinoma in fewer than 10% of cases, whereas 80% of patients with high-grade dysplasia develop oesophageal cancer within 6 years. Fifty percent of patients with histologically proven high-grade dysplasia are treated for cancer within the first two years of diagnosis (12). Therefore, patients with high-grade dysplasia have to undergo endoscopic or even surgical resection of the dysplastic area. Patients with low-grade dysplasia can also participate in an annual surveillance program.

Endoscopic ablations have some limitations. Retrospective analyses of our endoscopically treated patients show complete histological resection is achieved in only 85% of cases and there is a 30% recurrence rate within the first two years. A limited resection of the oesophagogastric junction and reconstruction with a jejunal interposition needs to be discussed for these cases and for patients with a mucosal or submucosal adenocarcinoma of the oesophagogastric junction. In our experience, the outcome of limited resection in these patients is very good. Even in patients with a T1b adenocarcinoma, who have lymph node metastases in approximately 20% of cases, limited resection results in a favourable prognosis, with a 5-year survival rate of up to 84% because the lymphadenectomy can be performed adequately (13). A retrospective analysis showed that a median of 19 lymph nodes were removed. These lymph nodes were localized in the greater and lesser curvature, along the celiac truncus, the hepatoduodenal ligament and

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Table I. *Stage-adjusted treatment of Barrett's metaplasia and carcinoma.*

Histology	Treatment
Dysplasia	
Low-grade	Endoscopic surveillance program of annual endoscopies with biopsies.
High-grade	1. Endoscopic ablation and annual examinations. 2. Limited gastroesophageal resection with complete resection of the intestinal metaplasia and jejunal interposition.
Carcinoma	
T1a	1. Limited gastroesophageal resection with complete resection of the intestinal metaplasia and jejunal interposition. 2. Endoscopic ablation and annual examinations.
T1b	1. Limited gastroesophageal resection with D2 lymphadenectomy and paraoesophageal and peribronchial lymphadenectomy (20% positive lymph nodes). 2. Abdomino-thoracic oesophagectomy with lymphadenectomy and reconstruction with a gastric tube.
T2	Abdomino-thoracic oesophagectomy with lymphadenectomy and reconstruction with a gastric tube.
T3, all N1 stages	Multimodal therapy with PET response and response prediction with customized microarray gene chips within scientific studies: neoadjuvant chemotherapy with cisplatin, 5-fluorouracil, leucovorin; following abdomino-thoracic oesophagectomy and reconstruction with a gastric tube.
T4; all M1 stages	Palliative chemotherapy, endoscopic stenttherapy, lasertherapy, cryotherapy

paraoesophageal up to the tracheal carina. In 100% of cases, it was possible to perform a complete resection of the tumour and the intestinal metaplasia, with a limited resection of the oesophagogastric junction. Postoperative morbidity is very low, at fewer than 5%, swallowing function is undisturbed, and the preserved stomach can maintain its tasks without any oesophageal reflux.

Treatment of Locally Advanced Barrett's Carcinoma

In 1996, Walsch and Henessy reviewed 55 patients undergoing primary resection for adenocarcinoma of the oesophagogastric junction. The median survival was 12 months, 3-year survival not even 10% (14). Meanwhile, philosophy of the treatment has changed towards a multimodal therapy consisting of neoadjuvant chemotherapy and following resection with a significantly better outcome. In patients that respond to chemotherapy, the 5-year survival rate reaches almost 70% (Figure 1) (15-19).

In responding patients, neoadjuvant chemotherapy results in downsizing and downstaging of the primary tumour, with a therefore higher probability of complete resection (20). The downsizing can be demonstrated with the use of computed tomography (CT) scans or endosonography at the beginning and the end of the therapy, or also in relation to the postoperative specimen. The downstaging can be demonstrated by histopathology. In up to 10% of all patients, a complete response without any vital tumour cells on histology ($ypT_0\ N_0$) can be detected as a sign of the best possible down staging result (17-19). In about 40% of patients, a good response rate with less than 50% vital tumour cells, disproportionately low numbers of involved lymph nodes, and reduced depth of invasion with scarring tissue surrounding the residual mucosal tumour, as the main prognostic factors, can be found. In our study, only 13% of responding patients have a positive lymph node status compared to the positive lymph node status in 80% of patients who did not participate in a multimodal therapy concept (14, 21). In a retrospective study, we showed that 44 out of 86 patients (51%) with an adenocarcinoma of the gastroesphageal junction, who underwent a primary oesophageal resection and reconstruction with a gastric tube, had lymph node metastasis in the postoperative histopathological work-up (22). However, after neoadjuvant chemotherapy, in about 50% of patients, only a minor response or no response was histopathologically detected after neoadjuvant chemotherapy with subsequent resection (21).

In order to plan an individually tailored multimodal therapy, it would be desirable to be able to differentiate responding from non-responding patients as early as possible. The comparison of conventional restaging modalities, such as endoscopy, endosonography and CT, to the histopathological response showed a low specificity (23). The interpretation of these examinations after neoadjuvant chemotherapy is often very difficult because of inflammatory alterations, tumour necrosis, scar tissue, oedema and differentiation from vital tumour (24). Early response evaluation with PET scan is possible because it assesses metabolism rather than morphology. With the correlation of a baseline PET scan and another after two weeks of chemotherapy, the response can be evaluated and individual multimodal therapy becomes possible. A decrease of more than 35% ^{18}F-fluorodeoxyglucose (FDG) uptake identifies the histological responders (25, 26). With this early response evaluation, it is possible to avoid chemotherapy-induced side-effects in patients that do not profit from chemotherapy,

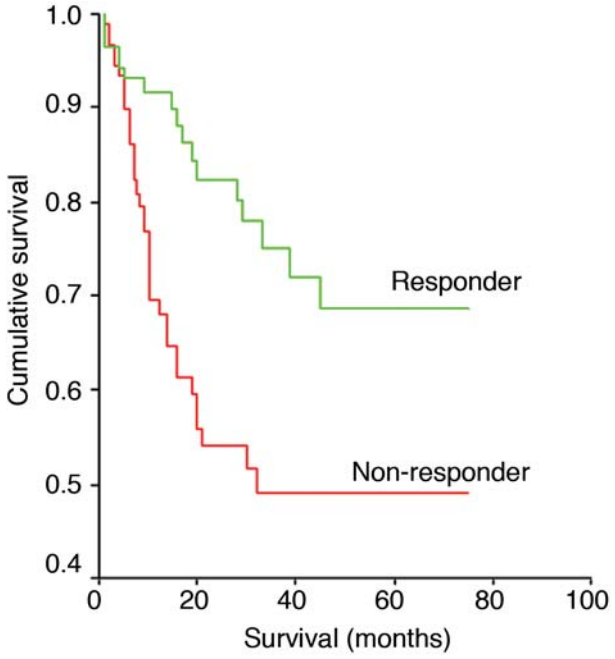

Figure 1. *Cumulative survival of patients with Barrett's carcinoma after multimodal therapy with neoadjuvant chemotherapy and following resection. Comparison of responders versus non-responders ($p<0.001$).*

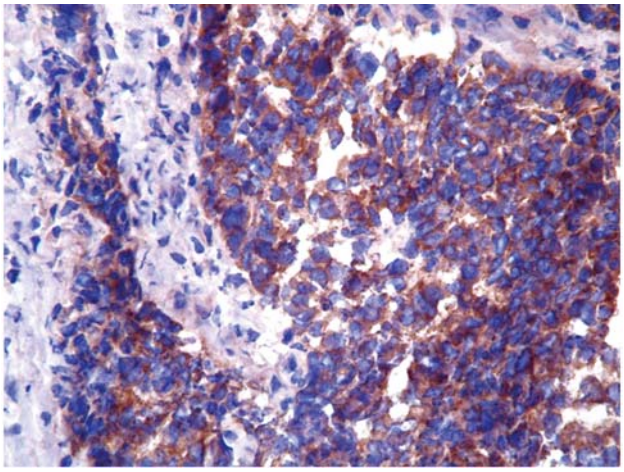

Figure 2. *Immunohistochemistry in this moderately differentiated Barrett's carcinoma demonstrates a strong expression of the ephrin receptor. This patient had a good chemotherapy response rate with fewer than 20% viable tumour cells, no lymph node involvement and an initial infiltration of the muscularis (T_2-category). The tumour is clearly delineated from the ephrin B3-negative connective tissue (original magnification ×40).*

and instead perform an early resection in these patients. Moreover, the early metabolic PET response is significantly correlated to long-term survival and can therefore differentiate patients with a good prognosis from those with an unfavourable prognosis very early.

A further advancement to the early PET response evaluation is a pretherapeutic response prediction from the time of the primary diagnosis, even before starting a neoadjuvant therapy (21). In order to characterize the differences between responding and non-responding tumours, our scientific group performed a microarray analysis (Affymetrix genechips U133 plus 2.0) of endoscopic biopsies of locally advanced Barrett's carcinoma, obtained within the primary clinical staging. All patients underwent neoadjuvant chemotherapy with cisplatin, 5-fluorouracil and leucovorin and subsequent abdominothoracic oesophageal resection. The postoperative histopathological work-up with staging according to the Union Internationale Contre le Cancer" (UICC) (27), grading and response evaluation according to Becker (28) was correlated to the microarray analysis results. Sixty-eight genes with at least two-fold differences in gene expression were identified (21). The genes encoded for the regulation of transcription, translation, receptors, cell cycle, cell-cellinteraction, the cytoskeleton, metabolism and protein synthesis (Table II). The strongest differences in down-regulated genes of responding patients were found for polyribonucleotide nucleotidyltransferase and transmembrane protein 5, and in up-regulated genes for tumour necrosis factor and ephrin B3 receptor. The high expression rate of the ephrin receptor in responding tumours was confirmed at the protein level with immunehistochemistry (Figure 2). High expression rates of the RNA and protein were significantly associated with response, depth of invasion and lymph node status ($p<0.001$; $p<0.001$; $p<0.001$ respectively). Therefore, the analysis of the ephrin B3 receptor as a new biomarker may help in differentiating responders from non-responders, even before starting chemotherapy, and may serve as a prognostic factor with regard to lymph node involvement.

The ephrin B3 receptor was identified in 1987 by its homologous sequence to the tyrosine kinase domain of a viral oncogene (29). The receptor is a transmembrane protein with an extracellular ephrin ligand-binding site and a cytoplasmic tyrosine kinase. The receptor group is divided into A and B receptors depending on the corresponding ligand. There are 9 A-type and 4 B-type ephrin receptors (30, 31).

In embryology, the ephrin B3 receptor plays an important role in the nervous system for axon guidance, development, cell intermingling and vasculature (30, 32). In the intestinal epithelium, it controls cell positioning (33). It restricts cell intermingling and allocates cell populations within the intestinal epithelium along the crypt-villus axis (34). Ephrinreceptor-ligand signalling provides cell positioning by repulsive clues in the intestinal epithelium. It was shown that it thus mediates compartimentalization and restricts the spreading of tumour cells into ephrin B1 ligand-positive territories (35, 36).

Table II. *k-Mean cluster of genes with at least 2-fold up- (left column) and down-regulation (right column) comparing responding versus non-responding oesophageal adenocarcinoma.*

Functional category	Chemotherapy	Response
	Increased gene expression	Decreased gene expression
Transcription/ translation/ protein synthesis	Enhancer of zeste homolog 1	Polyribonucleotide nucleotidytransferase
	Zinc-finger protein 395/ F-box protein 16	Cleavage and polyadenylation specific factor 2
	Cbp/p300-interacting transactivator	PNC1 protein
	Cytoplasmic polyadenylation element-binding protein 2	Tyrosyl-tRNA synthase
	DEAD box polypeptide 3, Y-linked	Zinc-finger protein 330
	DALR anticodon binding domain	
	Eukaryotic translation initiation factor 1A	
	Smcy homolog, Y-linked	
Receptor/channel/ membrane protein	EPH receptor B3	Transmembran protein 5
	Tetraspanin 7	FGFR1 oncogene partner
	Potassium inwardly-rectifying channel	
	Purinergic receptor P2Y, G-protein coupled	
	Mastermind-like 3 protein	
	Integral membrane protein 2A	
	Vasoactive intestinal peptide receptor 1	
Transcription factors/ nuclear proteins	Tumour protein p53 inducible nuclear protein 1	Homebox B9
	Chromosome 15 open reading frame 48	PHD finger protein 5A
	Jumonji, AT rich interactive domain 1B	SN ribonucleoprotein polypeptide A and G
	Yippee-like 3	Chromosome 5 open reading frame 22
		Kinesin family number 2C
		KIAA 0101
		H2A Histone family member Z
		Heterogenous nuclear ribonucleoprotein H3
Cell cycle/ cell devision	Septin 10	Cell devision cycle associated -2, -3, -8, -25
		Barren homolog 1
		Cyclin A2
		Cyclin-dependent kinase inhibitor 3
		Aurora kinase A
		ZW10 interactor
		Spindle pole body component 24 homolog
Cytoskeleton	Ankyrin repeat domain 29	Anillin, actin-binding protein
Metabolism/ enzymes	Lanosterol synthase	TACC 3
	Ubiquitin-conjugating enzyme	Mesoderm specific transcript homolog
	N-Terminal asparagine amidase	Acid phosphatase 1
	Homer homolog 2	
	Fatty acid-binding protein 1	
	Aldolase B	
	Monoamine oxidase A	
Protease/ protease inhibitors	MAP kinase-interacting serine/ threonine kinase 2	Proteasome (prosome, macropain) subunit, β-type
	Calpain small subunit 1	Proteasome (prosome, macropain) subunit, α-type
	MOB1, Mps one binder kinase activator like 2A	
	Spermidine/spermine N1-acetyltransferase	
Immune response/ inflammation	Tumour necrosis factor α-induced protein 8	MHC I polypeptide-related sequence B
	CD99 molecule	Interleukin 17 receptor B
		Small inducible cytokine subfamily E
Signal transduction	LRG1 leucine-rich α-2-glycoprotein 1	Transmembrane, prostate androgen-induced RNA.
	granulin	

The ephrin B3 receptor networks with the WNT signalling pathway, which stabilizes the β-catenin/transcription factor (TCF)-complex, which negatively controls the ephrin B3 receptor (34, 37). It was shown that mutational activation of the β-catenin/TCF-complex inversely controls the expression of ephrin B3 receptors in intestinal epithelial cells and leads to polyp formation as a first morphological step towards development of colorectal cancer (36). In the epithelial tumour cells, ephrin B3 signalling couples cell contraction with cell-to-cell-adhesion by promoting the recruitment of E-cadherin to the membrane. The compartmentalization of ephrin B3 receptor-positive Barrett's carcinoma was verified by

immunohistochemistry showing islets of immunohistologically positive carcinoma cells (Figure 2). This interaction of the ephrin receptor with its ligand shows the direct interaction of tumour cells in homing and the significance of the microenvironment concerning the depth of invasion and the development of lymph node metastases. As already mentioned, this correlation was shown statistically by comparing RNA expression and protein synthesis with the histopathological staging and with the receptor distribution as shown by immunohistochemistry.

In colorectal cancer, the function of the ephrin receptors could be described as having a similar impact on cell adhesion. In these cells, the stimulated ephrin receptor recruits disseminated E-cadherin to the membrane for cell-celladhesion (36). In Barrett's carcinoma, as yet, only a decrease in the expression of E-cadherin from columnar metaplasia to dysplasia and to oesophageal carcinoma has been detected, without any correlation to ephrin receptor expression. Further investigations concerning this topic need to be undertaken.

Nevertheless, response prediction from an endoscopic biopsy of the tumour seems to be a possibility in the near future using microarray analysis combined with immunhistolchemistry.

Conclusion

The treatment of Barrett's carcinoma needs to be stage adjusted, with surgical treatment directly for patients with high-grade dysplasia. In order to differentiate dysplasia from mucosal or submucosal carcinoma, endoscopy with biopsies and endosonography are the most important examinations. In cases of locally advanced carcinoma, PET/CT and endoscopic biopsies for response prediction and evaluation should be performed. In cases requiring multimodal therapy, we recommend treatment within a scientific study setting with analysis of response. Further data concerning a response prediction with a customized gene chip (with our 68 most differentially expressed genes) and the corresponding immunohistochemistry, as the most promising approach in our opinion, need to be collected. Some questions in this context still remain for further investigation. In the treatment of non-responding patients, intensified chemotherapy with antibody therapy or other chemotherapeutic agents vs. primary resection requires further discussion. Additional radiotherapy is under consideration for these patients.

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