

BIOMARKERS IN OESOPHAGOGASTRIC CANCERS

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Abstract

There is global recognition that there is an increasing need to generate predictive and prognostic biomarkers in various malignancies. Personalised medicine is progressing in certain streams of medical oncology yet remains an elusive goal in oesophagogastric cancer. This article reviews the role of F-18 fludeoxyglucose PET and various molecular methodologies in exploring potential biomarkers in the management of oesophageal and gastro-oesophageal cancers.

Biomarkers are defined as objectively measurable parameters that predict a biological state or behaviour, such as response to treatment. A prognostic biomarker is a term used to describe the likely course of disease in any one individual. This historically meant an individual who had not been treated before but this definition is increasingly complicated by the use of post-operative therapies, which alter the course of disease. Predictive biomarkers are defined as markers that can identify subpopulations of patients that are likely to respond to a given treatment. This allows for a tailoring of treatment as a specific treatment regimen is chosen for any one individual, as they are more likely to respond to it than the alternatives. Biomarkers are sought in pathology specimens, in blood collected from the affected patient and even using radiological investigations, to document changes that may predict a patient's progress.

Biomarker research has developed in an attempt to shorten clinical trial duration, but also to provide endpoints that have a biologic relevance to the clinical intervention under study. A pathologic biomarker that is often used as a surrogate endpoint in induction therapy trial design is the "percent pathologic response" in the resected primary tumour after induction therapy has been given. A major histological response to pre-operative chemotherapy or chemoradiotherapy ($\leq 10\%$ residual tumour with the remainder of the lesion replaced by fibrosis including pathological complete response) has been shown to be an independent prognostic factor.¹ Several studies have shown no significant difference in survival between patients with a pathological complete response or $<10\%$ residual tumour.^{2,3}

In this article, we will review the current status of biomarkers in oesophagogastric cancer, reporting on the evidence to date in oesophageal adenocarcinoma and gastro-oesophageal carcinoma.

PET as a biomarker in operable oesophagogastric cancers

There is a considerable body of PET data concerning oesophageal adenocarcinoma, particularly regarding tumours in the gastro-oesophageal junction. The only biomarker that has undergone significant investigation is F-18 fluorodeoxyglucose (FDG) PET.

FDG PET as a prognostic biomarker in the untreated patient reflects the intrinsic biology of the patient's cancer and is best examined in patients who have no induction therapy but proceed directly to surgery, because of the absence of confounding interventions. Patients with 'early stage' disease are the ones most likely to benefit from such an approach. Early stage oesophageal cancer has been defined as T1-2 N0 M0. The stage can only be truly confirmed pathologically, but defining the stage on a resected specimen prohibits the patient from potentially benefiting from an induction therapy approach followed by surgery. Rizk et al examined the utility of FDG PET in patients with surgically resected early-stage oesophageal cancer without induction therapy.⁴ In this analysis of 50 patients, a median standardised uptake value (SUV) of FDG by the tumour (SUV=4.5) was arbitrarily selected to stratify patients as high or low risk. Those patients with a higher than median SUV_{max} had a statistically significantly shorter three year survival of 57% compared to the 95% seen in those patients with a lower than median SUV. The patients with a low SUV (<4.5) had a 90% chance of having a T1-2 tumour (24/25) compared to 60% (15/25) in the high SUV group. The incidence of an involved N1 or M1a node was 8% (2/25) in the low SUV group but was 48% (12/25) in the high SUV group. In the 32 patients who were classified pathologically as early stage disease (T1-2N0M0), 22/32 (69%) were in the low SUV group and 10 (31%) were in the highmax SUV group. In the 32 with pathologic T1-2N0 M0 disease, low SUV (<4.5) had a significantly better survival ($p=0.023$). The implications of this data are that patients with a high SUV should be considered at high risk of recurrence and death. This allows for further preoperative risk stratification beyond the traditional TNM staging and may allow for a more tailored approach to subsequent treatment.

Oesophageal cancer and early FDG PET response

While histological response is a reasonable surrogate for survival in oesophageal adenocarcinoma, this end-point is obtained after the completion of the selected pre-operative therapy and surgery. Thus, non-responders to pre-operative therapy are only identified when the options to change therapy are limited and can only be given post-operatively. Given only 50-60% of patients are fit for post-operative chemotherapy,⁵ this option has limited value. The prospective

Table 1: Summary of Australian trials of targeted therapy of oesophagogastric cancers.

Marker	Function	Potential biomarker role
VEGF	Involved in development and maintenance of a vascular network that may facilitate tumour growth and metastasis.	Higher VEGF index in gastro-oesophageal cancer correlates with poorer histopathological response to neoadjuvant chemoradiotherapy; ² polymorphism VEGF 936C>T correlated with median disease-free survival and combined with PET scans was independent prognostic factor for clinical and histopathological response. ¹²
COX-213-16	Rate-limiting enzymic conversion of arachidonic acid to prostaglandins, is induced by cytokines, growth factors and oncoproteins, and regulates tumour onset and progression, metastases, angiogenesis and resistance to chemotherapy. ¹⁷	mRNA levels proportionate through sequence from Barrett's metaplasia to dysplasia to oesophageal adenocarcinoma; ¹⁸⁻²¹ higher levels associated with greater resistance to apoptosis; ²² high intratumoural mRNA and protein levels in oesophageal cancer (40% oesophageal adenocarcinoma) were associated with less response to neoadjuvant chemoradiotherapy. ²³
p53	Involved in cell cycle regulation, apoptosis and DNA repair.	Increased in progression toward oesophageal adenocarcinoma and oral squamous cell carcinoma; mutations in 40-50% oesophageal adenocarcinoma; ^{12,24} correlates with low rates of pathological complete response and worse disease-free survival and overall survival, ²⁶ with after neoadjuvant chemoradiotherapy; others failed to show gene or protein levels reflecting response rates. ²⁷⁻³⁰
Survivin	Plays a central role in (dys)-regulation of apoptosis; survivin has also been implicated in cell-cycle regulation and tumour angiogenesis. ³¹	mRNA levels proportionate through sequence from Barrett's metaplasia to dysplasia to oesophageal adenocarcinoma; ³² levels reduced after neoadjuvant chemoradiotherapy and failure to do so was associated with worse prognosis and only minor histopathological response. ^{33,34}
NF-κβ	Sequence specific transcription factor acting as gatekeeper for cell survival, proliferation, invasion and metastasis.	Associated with aggressive pathological features when overexpressed like perineural and lymphovascular invasion, metastases ³⁵ ; absence of expression associated with response to neoadjuvant chemoradiotherapy. ^{36,37}
HIF-1α	A transcription factor linked to genes involved in response to cellular hypoxia including vasculogenesis and angiogenesis, metabolism, vasodilation, cell migration, signalling and cell fate decisions.	Correlated with tumour aggressiveness and prognosis in oral squamous cell carcinoma in far eastern population ³⁸⁻⁴⁰ ; not confirmed in European population.

Key: VEGF – vascular endothelial growth factor; COX-2 – cyclo-oxygenase 2; NF-κβ – nuclear factor κβ; HIF-1α – hypoxia-inducible factor 1α.

study by Ott et al demonstrated that a ≥35% reduction in pre-treatment primary tumour SUVmax found on a second FDG PET scan on day 14 ('early metabolic response') and after the first cycle of paclitaxel, cisplatin and 5-Fluorouracil (5-FU) chemotherapy (21/56 patients received CF alone), could predict response to neoadjuvant chemotherapy and survival after resection.⁶ All patients in the study went on to receive another two cycles of chemotherapy without radiotherapy. Early metabolic response was associated with a major histological response in 44% of patients compared with 5% for non-responders. Similarly, an early metabolic response to therapy was an independent prognostic factor for progression-free survival (PFS).⁶ Subsequently, Lordick et al reported the only clinical study that has modified

therapy based on an early metabolic response.⁷ The MUNICON trial studied 110 patients with oesophageal adenocarcinoma. Patients that showed an early metabolic response to the first cycle of pre-operative chemotherapy then received a further five cycles of therapy then resection with two-year survival of 75%. Metabolic non-responders on the day 14 PET scan received no further chemotherapy and went directly to surgery resulting in a two-year survival of 60% and no histological responses. MUNICON II added radiotherapy to these non-responders, using the same chemotherapy regimen.⁸ The result was improved response rates. However, survival rates remained poor, suggesting that FDG PET has utility as a prognostic marker, but in this circumstance was a poor predictive marker.

Rizk et al reported the Memorial Sloan-Kettering Cancer Centre experience of the efficacy of a baseline FDG PET to predict response and survival in patients with gastro-oesophageal cancer undergoing chemoradiotherapy induction.⁹ They found that although there was no difference in survival, the patients with the higher baseline SUV had higher response rates on the post-induction specimen. The finding that a high SUV at baseline predicts for pathological response rates was independently confirmed by Lordick.⁷ The interpretation made by Rizk et al was that the patients with a low initial baseline SUV had an inherently better survival from a biological perspective, which was not altered by the addition of induction therapy.⁹ The patients with the higher SUV initially had a worse prognosis that was improved significantly by the addition of induction therapy. Rizk et al suggests that the FDG PET can predict those high risk patients who are most likely to benefit most from induction therapy.⁹

The above data suggests FDG PET, when performed with due consideration of the underlying principles, including imaging on the same camera, reconstructing the data with a consistent algorithm and using a consistent time post-injection provides prognostically significant data worthy of consideration as a valid biomarker in oesophageal cancer. In Australia, the DOCTOR trial is exploring the role of adjuvant chemotherapy +/- radiotherapy based on a poor early response to neoadjuvant chemotherapy.¹⁰

Molecular approaches for improving our understanding of oesophagogastric cancers

Research studies aimed at identifying prognostic biomarkers in oesophagogastric cancers have generally used a candidate gene or marker approach in the tumour in order to determine whether an association exists with survival, American Joint Committee on Cancer stage or response to chemotherapy or chemoradiotherapy (reviewed in Lagarde, 2007).¹¹ Although there are potential biomarkers and many demonstrate prognostic value, subsequent replication of these findings is lacking (table 1). Since several molecular alterations can act together to influence tumorigenesis, it is unlikely that a single biomarker alone can accurately predict survival. The following are various methods exploring the evidence for predictive biomarkers in oesophageal adenocarcinoma.

Gene expression profiling

Gene expression profiling is the measurement of gene expression in a given sample, often thousands at once, to give a snapshot of cellular function. In this way, it is hoped that an individual tumour can be characterised to better target investigation of biological pathways and ultimately, facilitate drug design. This has been demonstrated in breast cancer with some signatures predicting survival and response to chemotherapy.⁴¹⁻⁴³ It is less well advanced in oesophagogastric cancers.

A number of studies have explored the use of mRNA expression profiling to predict survival and/or treatment response in individual oesophageal adenocarcinoma patients, with conflicting results. Real time pathological complete response on 38 pre-treatment endoscopic biopsy sections focused on expression of 5-FU, platinum and taxane related genes and found that high expression levels of MTHFR,

CALD1 and MRP1 are related to response and survival.⁴⁴ Langer et al have since shown that high pre-treatment levels of MRP1 and TS in oesophageal adenocarcinoma, are associated with a poor response to chemotherapy.⁴⁵ Chemotherapy response was also investigated in a cohort of 47 patients with oesophageal adenocarcinoma in which 86 genes were differentially expressed between responders to neoadjuvant chemoradiotherapy and non-responders.⁴⁶ The authors showed a significant correlation between the Ephrin B3 receptor and response, but these results are yet to be externally validated. This tyrosine kinase receptor has a role in morphogenesis, tumorigenesis and metastatic potential, angiogenesis and tumour vasculature in the gastrointestinal tract.⁴⁷ Similarly, Schneider et al showed that DPD, ERCC1, TS, GSPi, HER2 and EGFR mRNA expression was downregulated in response to neoadjuvant chemoradiotherapy for oesophageal cancer.⁴⁸

Luthra et al reported on an oligonucleotide microarray performed on pre-treatment endoscopic biopsies from 19 patients (16 adenocarcinoma, two SCC, one adenosquamous) prior to trimodal therapy.⁴⁸ Unsupervised clustering was used to identify associations in the microarray according to response, however no statistically significant associations with gene expression were identified. This may be due to the underlying assumption of the unsupervised clustering method which assumes there is a pattern, even when data is truly random.

Duong et al also conducted a microarray study which analysed the expression profiles in 46 patients (25 with oesophageal adenocarcinoma).⁴⁹ They were able to identify a 32-gene classifier to predict chemoradiotherapy response for SCC, but a gene signature predictive for oesophageal adenocarcinoma was not identified.

Recently, Peters and colleagues have reported a four-gene prognostic signature (DCK, PAPSS2, SIRT2 and TRIM44) for oesophageal adenocarcinoma and gastro-oesophageal cancer (table 2).⁵⁰ The discovery phase was undertaken using a 44k cDNA microarray for 75 patients with oesophageal adenocarcinoma of varying stage, and externally validated using tissue microarrays constructed from a separate oesophageal adenocarcinoma and gastro-oesophageal patient cohort (n=371). While the mixture of stages and treatment types may confound the results, the study is of large size and is the only microarray-based biomarker investigation conducted in oesophageal adenocarcinoma that has demonstrated external validation of the prognostic signature.

Table 2: Five-Year Survival Rates Based on the Four-Gene Signature Score.

Number genes dysregulated (of four gene signature)	Five year survival % (95% CI) ^a
0	58 (36-80)
1-2	26 (20-32)
3-4	14 (4-24)

^a *p* = 0.013. Key: CI = confidence interval.

DNA copy number variations in oesophageal adenocarcinoma

Copy number variations are a form of structural variation where there is an abnormality in one or more sections of DNA within a cell. The degree of changes in chromosomal numbers within a cell,⁵¹ the presence and degree of DNA damage and the proportion of flow cytometric-sorted biopsy fractions that display 4n DNA content,^{51,52} have been proposed as markers of the progression of Barrett's Oesophagus to oesophageal adenocarcinoma to be used in conjunction with dysplasia status. Amplifications in several genomic regions can lead to the increased activity of oncogenes (eg. MYC), which promote autonomous cell growth. In order to profile whole genome DNA copy number changes in oesophageal adenocarcinoma, several groups have employed array-based comparative genome hybridisation studies or loss of heterozygosity microsatellite-based methods. The most frequently reported regions of loss are 4q, 5q, 9p, 17p, 18q and Y, while frequent gains are on 7p, 8q and 20q.

Several other studies have looked for gene-based DNA copy number changes in oesophageal adenocarcinoma and found that FHIT, CDKN2A and TP53 are frequently lost, while MYC, MYBL2 and ERBB2 are gained.^{53,54} High resolution DNA copy number screening using SNP arrays in oesophageal adenocarcinoma tumours have found copy number events to be common, averaging 76 (range, 5-152) per tumour.⁵⁵ Losses and gains averaged 20 (range, 1-62) and 16 (range, 1-54) per tumour respectively, and copy neutral loss of heterozygosity events averaged 41 (range, 3-75) per oesophageal adenocarcinoma.⁵⁵ More high resolution studies are still required, particularly those linked to clinical trial outcomes.

DNA methylation

Methylation of DNA within CpG islands, or sections of the genome with high levels of the DNA building blocks cytosine and guanine, are being found to occur in a growing number of genes to varying degrees in human cancers, including oesophageal adenocarcinomas.⁵⁶ In normally differentiated, non-neoplastic tissues these genes are mainly unmethylated. It is thought to be a critical mechanism for tumour suppressor gene silencing and inactivation.⁵⁷ Circulating tumour cells with methylated CpG islands have been proposed as a prognostic indicator and for tumour detection in colorectal cancer.⁵⁸

A retrospective analysis of CpG island hypermethylation was assessed in 11 candidate genes in pre-treatment tumour specimens (oesophageal adenocarcinoma 23, oral squamous cell carcinoma 12).⁵⁹ The patients received neoadjuvant trimodal therapy. A lower number of methylated genes per patient (1.2 versus 2.4, $p=0.026$) was associated with pathological complete response.

MethylLight assays have been used to identify methylation in the promoter regions of the CDKN2A, HPP1 and RUNX3 genes, and to distinguish Barrett's Oesophagus tissue at risk of progression to oesophageal adenocarcinoma.⁶⁰⁻⁶² Frequent differences in the methylation profiles for nine cancer related genes in oesophageal adenocarcinoma, compared with normal squamous epithelium, have also

been demonstrated,⁶³ as has aberrant methylation of the E-cadherin promoter, which seems to be a common cause of inactivation in adenocarcinomas.⁶⁴

These data suggest that methylation differences may be suitable candidates for prognostic biomarkers in oesophagogastric cancers.

DNA point mutations in oesophageal adenocarcinoma

Somatic mutations are a well recognised phenomenon in tumour biology, often in combination with changes in DNA copy number. TP53 loss of heterozygosity and mutations seem to be relatively early events in neoplastic progression in Barrett's Oesophagus and TP53 mutation frequency estimates range from 35-69% in oesophageal adenocarcinoma.^{55,65-68} Although allelic loss of CDKN2A appears to be common, point mutations appear to be rare.⁶⁹⁻⁷¹ A number of other candidate genes have been explored for somatic mutations in oesophageal adenocarcinoma, including APC, CDH1, CTNNB1, EGFR, FHIT, BRAF, KRAS, TGFB, PIK3CA and PIK3CB.

Recently, Boonstra et al demonstrated that the CDH1 GA/GA phenotype was associated with reduced survival and conversely, the MDM2 T/G phenotype with improved disease-free survival.⁷² They suggest that the individual differences in germ-line DNA have an impact on disease-free survival in oesophageal adenocarcinoma.

In general however, the reported frequency of somatic mutations identified in the genes studied in oesophageal adenocarcinoma appears to be low. Despite frequent allelic loss of 5q on which APC resides, a very low rate of APC mutations was described in oesophageal cancers.⁷³⁻⁷⁶ Similarly, mutations of the E-cadherin,⁷⁷ FHIT,⁷⁸ CTNNB1,⁷⁸ TGFB,⁷⁹ and EGFR genes,^{80,81} were rarely described in adenocarcinomas. A recent report identified activating BRAF mutations in 2/19 tumours and KRAS mutations in 4/19 tumours, suggesting that the disruption of the Raf/MEK/ERK (MAPK) kinase pathway is frequent in oesophageal adenocarcinoma,⁸² findings supported by other studies.^{83,84}

In summary, only a handful of selected genes have been investigated for somatic mutations in oesophageal adenocarcinoma. The majority of these genes have been selected based on evidence from other cancer types. Although the lack of somatic mutations found in these genes might suggest that the frequency of somatic mutation in oesophageal adenocarcinoma is low, an alternative hypothesis is that a different set of genes is mutated in oesophageal adenocarcinoma, which in part could account for the different disease course and poor survival. The landscape of human genetics is rapidly changing with the advent of massively parallel sequencing technologies.⁸⁵ The first cancer genomes to be published have revealed thousands of novel somatic mutations and implicated new genes and processes in tumour development and progression.⁸⁶ Next generation sequencing is particularly appealing because it can detect the full spectrum of genetic variants in cancer, which could allow for a further differentiation which reflects the phenotype. It may also allow for the identification of novel therapeutic targets for future investigation.

Drug metabolism genes

Iqbal et al examined various genes in a cohort receiving XELOX and cetuximab as first-line treatment of gastric and gastroesophageal junction adenocarcinoma.⁸⁷ The genes chosen were related to 5-FU (TYMS, MTHFR) and oxaliplatin (ERCC1, XPD, GSTP1) metabolism, and human epidermal receptor signalling (EGF, HER2, COX2, FCgR2A, FCgR3A). It has shown a statistically significant association between SNPs EGF A+61G rs4444903 and GSTP1 Ile105Val rs1695 and response rate. There was a significant association with MTHFR 1298 A1298C rs1801131 and the overall survival ($p=0.044$).

ERCC1 gene expression levels have been shown to be predictive of response and survival to platinum-based chemotherapy.^{88,89} Increased ERCC-1 mRNA expression may be an indicator for non-responsiveness to neoadjuvant CDDP-based chemotherapy.⁹⁰ Conversely, low intratumoral expression of ERCC1 correlated significantly with better response to neoadjuvant chemoradiotherapy, even though overall survival could not be evaluated due to short follow-up.

Conclusion

A number of biomarkers have been reported in oesophagogastric cancers. While they have been shown to be variously prognostic or predictive in response to treatment, none have been prospectively validated and most are in small patient populations. Integrating these findings into prospective trials will hopefully herald their use in everyday clinical practice and thus, improve the management of oesophagogastric cancer into the future.

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