The metabolic marker tumour pyruvate kinase type M2 (tumour M2-PK) shows increased expression along the metaplasia–dysplasia–adenocarcinoma sequence in Barrett’s oesophagus

K Koss, R F Harrison, J Gregory, S J Darnton, M R Anderson, J A Z Jankowski

Background: Proliferating and tumour cells express the glycolytic isoenzyme, pyruvate kinase type M2 (M2-PK). In tumours cells, M2-PK usually exists in dimeric form (tumour M2-PK), causing the accumulation of glycolytic phosphometabolites, which allows cells to invade areas with low oxygen and glucose concentrations.

Aims: To investigate the expression of tumour M2-PK during the metaplasia–dysplasia–adenocarcinoma sequence of Barrett’s oesophagus, and to assess the prognostic usefulness of tumour M2-PK in oesophageal cancer.

Materials/Methods: One hundred and ninety cases selected from the histopathology archives as follows: 17 reflux oesophagitis, 37 Barrett’s oesophagus, 21 high grade dysplasia, 112 adenocarcinomas, and three control tumours. Sections were stained immunohistochemically with antibody to tumour M2-PK.

Results: Tumour M2-PK was expressed in all cases, and increased cytoplasmic expression was seen with progression along the metaplasia–dysplasia–adenocarcinoma sequence. All cases of adenocarcinoma showed 100% staining so that tumour M2-PK was not a useful prognostic marker.

Conclusions: Tumour M2-PK is not a specific marker of Barrett’s adenocarcinoma, but may be important as a marker of transformed and highly proliferating clones during progression along the metaplasia–dysplasia–adenocarcinoma sequence.

Abbreviations: M2-PK, pyruvate kinase type M2; PK, pyruvate kinase.
driving forces behind the maintenance and neoplastic progression of Barrett’s oesophagus, but also to enable translational research into treatment. With this in mind, we sought to characterise the expression of the tumour M2-PK marker of proliferation in Barrett’s oesophagus, Barrett’s dysplasia, and adenocarcinoma.

MATERIAL AND METHODS

Tissue samples
One hundred and ninety cases were selected from the histopathology archives as follows: 17 cases of reflux oesophagitis, 37 cases of Barrett’s intestinal metaplasia, 21 cases of high grade dysplasia in Barrett’s mucosa, and 38 cases of adenocarcinoma arising in Barrett’s oesophagus. An additional 74 cases of oesophageal adenocarcinoma with well documented survival data were selected from the oesophageal laboratory of Birmingham Heartlands Hospital. The local research ethics committees approved our study.

Immunohistochemistry
Formalin fixed, paraffin wax embedded, 3 μm thick tissue sections were subjected to a heat mediated antigen retrieval

Figure 1 (A) Negative control, no primary antibody in a case of colon cancer. (B) Positive control of colon cancer: all cells of the malignant glands show strong cytoplasmic positivity. (C) Normal small bowel showing strong staining of enterocyte cytoplasm. (D) Normal large bowel showing patchy strong staining of crypt cells. (E) Severe reflux oesophagitis. The lower half of the squamous mucosa shows strong cytoplasmic staining. (F) Barrett’s oesophagus. There is patchy strong cytoplasmic staining in groups of cells, whereas others are negative (cf normal small bowel). (G) Barrett’s dysplasia. Strong cytoplasmic staining of runs of surface epithelium and glands, whereas some groups are clearly negative. (H) Barrett’s adenocarcinoma. Widespread strong cytoplasmic staining of tumour cells.
Histological assessment

Staining was assessed with regard to intensity on a semiquantitative scale of 0, +, and ++ (no staining, weak staining, and strong staining, respectively). The proportion of cells stained was also assessed semiquantitatively as follows: 0, up to 30%, 30–60%, and 60–100%. A note of the location of the staining within the cells and of any staining patterns was also made.

RESULTS

Controls

Negative controls were uniformly negative (fig 1A). The positive tissue controls all showed staining. The three cases of adenocarcinoma of the colon were strongly positive (+++) in the cytoplasm of almost 100% of the tumour cells (fig 1B). Normal small bowel showed strong staining of the cytoplasm of the enterocytes (fig 1C), but normal large bowel showed only patchy staining of crypt cells (fig 1D).

Test groups (table 1)

All 17 cases of reflux oesophagitis showed positive (+++) staining in the cytoplasm of all cells in the lower third of the squamous epithelium. Occasional accentuation of the basal layer was noted, and occasional nuclear staining was seen. In more severe cases of oesophagitis, the lower half or even lower two thirds of the squamous mucosa showed strong cytoplasmic staining (fig 1E).

In three of the 37 cases of Barrett’s metaplasia 100% of the cells stained, 16 cases showed more than 60% positivity, 13 cases showed 30–60% positivity, and five cases showed less than 30% positivity. In these samples, the intestinal mucosa goblet cells were negative and it was both the intestinal absorptive cells and the mucous secreting cells that were positive. The variation in staining might result from the variable proportions of these two cell types. Some nuclear staining was occasionally noted (fig 1F).

All 21 cases of high grade dysplasia showed positive (+++) staining. In 19 of these cases more than 60% of cells were positive, and two cases showed 30–60% positivity. Where dysplastic cells were seen along the surface of the mucosa, staining was the most pronounced in these cells. Some cases showed continuous runs of positively stained cells interspersed with occasional groups of negatively stained cells (fig 1G).

All 112 cases of adenocarcinoma were positive (+++), with almost 100% of the cells staining (fig 1H). Nuclear staining for tumour M2-PK was seen in some sections of adenocarcinoma and in some inflammatory and endothelial cells.

Two independent assessors graded the staining and their counts were compared. The correlation coefficient (R = 0.85; p < 0.01) indicated that interindividual reproducibility was good.

DISCUSSION

Progression to adenocarcinoma from Barrett’s oesophagus is thought to be associated with the intestinal metaplastic type of Barrett’s oesophagus, which contains goblet cells. At a molecular level, expression of the oncogene products p53, p16, and adenomatous polyposis coli, among others, has been shown to be associated with malignant progression, although these markers are not accurate predictors of patient survival.11 In addition, some high risk individuals with a strong family history have E-cadherin mutations4 and interleukin 1β polymorphisms,12 which could provide an objective basis for screening. More recently, the importance of cyclooxygenase 214 and tumour necrosis factor α in the process of neoplastic progression has been recognised, with the potential to inhibit their actions pharmacologically.

There is still a need to find markers that could help in stratifying patients with Barrett’s oesophagus into high and low risk groups for targeted surveillance. Recently published data on tumour M2-PK concentrations in EDTA plasma samples from patients with oesophageal cancer showed that this marker was raised at least as often as the carbohydrate antigen 72-4 and substantially more frequently than carbohydrate antigen 19-9 or carcinoembryonic antigen.15 Tumour M2-PK was the best of these four markers at discriminating between patients with localised oesophageal cancer and a non-malignant control group. A smaller study published earlier16 suggests that tumour M2-PK may be a valuable marker for the detection of gastrointestinal cancers in general. Therefore, we undertook a simple immunohistochemical study to look at the distribution of the tumour metabolic marker tumour M2-PK in Barrett’s oesophagus, Barrett’s dysplasia, and adenocarcinoma.

<table>
<thead>
<tr>
<th>Tissue type (number of cases)</th>
<th>Staining intensity (cytoplasmic)</th>
<th>Proportion of cells positive %</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0</td>
<td>+</td>
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<tr>
<td>Reflux oesophagitis (17)</td>
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<td>0</td>
</tr>
<tr>
<td>Barrett’s metaplasia (37)</td>
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<td>0</td>
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<tr>
<td>Barrett’s dysplasia (21)</td>
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<td>0</td>
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<tr>
<td>Adenocarcinoma (112)</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Control tumours (3)</td>
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Lower 1/3-1/2 of mucosa
Oesophagitis squamous epithelium expressed tumour M2-PK, with an increased depth corresponding to more severe oesophagitis change (fig 1E). All cases of Barrett’s metaplasia expressed tumour M2-PK in their cytoplasm. Staining was variable, ranging from less than 30% cells positive to 100% positive, with both the intestinal absorptive cells and mucus secreting cells being positive and goblet cells negative. In the dysplastic cases, there was also variability in the proportion of stained cells, but a greater proportion of cells was stained overall (fig 1G). The variation in staining could be the result of the variable proportions of cell types. Alternatively, we must consider the issue of clonality: in Barrett’s mucosa there are probably multiple clones of metastatic cells, which are selected out along the metaplasia–dysplasia–adenocarcinoma sequence. In metaplasia and dysplasia, it is possible that the expression of tumour M2-PK reflects clones of cells within the epithelium with a metabolic switch leading to abnormally high anaerobic metabolism and enhanced neoplastic characteristics, such as increased proliferation and invasive potential. In all the cases of adenocarcinoma in Barrett’s oesophagus, almost 100% of the tumour cells stained strongly (fig 1H). The nuclear staining seen in some of our adenocarcinoma sections has been noted by other investigators, although currently a physiological explanation is lacking.

“There is still a need to find markers that could help in stratifying patients with Barrett’s oesophagus into high and low risk groups for targeted surveillance”}

The expression of Ki67 and the accumulation of p53, as measured by immunocytochemistry, have been reported to be only very weak markers of cancer risk in Barrett’s oesophagus. Therefore, we thought that there would be an association between the coexpression of these molecules and M2-PK, but that this association data would not provide definitive evidence that M2-PK is useful clinically. We found that the expression of tumour M2-PK increased as the Barrett’s metaplasia–dysplasia–adenocarcinoma sequence progressed. Because all cases of adenocarcinoma were almost homogenously stained, we recognise the limitations of tumour M2-PK as a prognostic marker for patient survival. However, tumour M2-PK is a common marker of neoplasia (vide infra) and, although not specific for Barrett’s adenocarcinoma, it may help to draw the pathologist’s attention to specific areas of tissue. Tumour M2-PK does indicate the extent of the “stress response” during mucosal inflammation in Barrett’s metaplasia. As such, these data provide additional information of the cell types at risk of oxidative stress, in addition to the early and sustained tissue damage and resultant clonal evolution of adaptive cells.

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REFERENCES