Allelic Imbalance on Distal 7q (7q36.1-q36.3) in Gastric Cardia and Oesophageal (Barrett’s) Adenocarcinoma

KEES J. VISSERS¹, WINAND N.M. DINJENS¹, PETER H.J. RIEGMAN¹, HUGO W. TILANUS² and HERMAN VAN DEKKEN¹

¹Department of Pathology, Josephine Nefkens Institute, Rotterdam; ²Department of Surgery, Erasmus University Rotterdam, P.O. Box 1738, 3000 DR, Rotterdam, The Netherlands

Abstract. Background: Oesophageal (Barrett’s) and gastric cardia adenocarcinomas are cancers arising at and around the gastro-oesophageal junction. The prognosis is poor, since detection is usually at a late stage and metastatic spread occurs early. Materials and Methods: We investigated the 7q region with a set of 5 polymorphic markers spanning 7q36.1-q36.3 in 33 Barrett-related carcinomas. In addition, 40 gastric cardia cancers were investigated to compare the pattern of imbalance at these loci. Results: Overall, the number of allelic loss was higher in Barrett’s cancers than in gastric cardia carcinomas (p=0.04). In Barrett’s adenocarcinomas, imbalance varied from 28% to 45% (of informative cases) with the highest prevalence at marker D7S483. In gastric cardia cancers, loss ranged from 12% to 27% (of informative cases), being most frequent at marker D7S3037. The difference between oesophageal and gastric adenocarcinomas was highest for polymorphic marker D7S483 (p=0.05). Conclusion: Marker D7S483 can aid in discriminating oesophageal (Barrett’s) and gastric cardia carcinomas. Further, this region possibly harbours cancer gene(s) involved in Barrett-related adenocarcinomas.

Over the past two decades, the incidence of adenocarcinoma of the oesophagus and gastric cardia has increased at a rate exceeding that of any other cancer. The survival rates of these cancers, that arise at and around the gastro-oesophageal junction (GEJ), is poor (1). First, these tumours show, in general, aggressive behaviour and metastasize early. Secondly, most cancers are diagnosed at a late stage and lymphatic or hematogeneous spread has already occurred. Clinical observations suggest that GEJ adenocarcinomas behave in similar fashions (2-4). Genetic and cell biological analyses of Barrett-related oesophageal adenocarcinoma and gastric cardia adenocarcinoma revealed an overlapping spectrum of genomic alterations (5-8). Recently, we reported frequent allelic imbalance of 7q32.3-q36.1 during tumourigenesis in Barrett’s esophagus (9). In the present study, we aimed at narrowing down the critical region of loss in Barrett-related cancers. Further, we investigated a series of gastric cardia carcinomas with the same panel of polymorphic markers and compared the distribution of allelic loss on distal 7q.

Materials and Methods

We retrieved DNA’s of 33 oesophageal (Barrett’s) and 40 gastric cardia adenocarcinomas from formalin-fixed (n=61) and fresh-frozen (n=12: 6 of each tumour site) patient material and mouse xenografts. A cancer was termed oesophageal adenocarcinoma if the centre was clearly situated in the distal oesophagus with or without specialised Barrett’s epithelium. Also, cancers at the GEJ in the presence of Barrett’s epithelium were considered oesophageal adenocarcinoma. A tumour was designated gastric cardia adenocarcinoma if the centre was located at the junction in the absence of Barrett’s mucosa, or if the centre of the tumour was seen in the proximal stomach. After isolating the DNA by standard procedures, LOH analysis was performed with a set of 5 polymorphic markers. The polymorphic markers were selected from the National Center for Biotechnology Information (http://www/ncbi.nlm.nih.gov/genemap/) and Genome Data Base databanks (http://gdbwww.gdb.org/) based on heterozygosity frequency, as well as coverage and flanking the region of interest. The location of the primers was determined by the most recent draft sequence (http://genome.ucsc.edu/). New primers were designed to shorten amplicons for markers larger than 200bp, since this might hamper amplification of DNA isolated from formalin-fixed, paraffin-embedded tissue. The markers are listed in Table I.

The PCR reaction mixture (15 µl) contained 1.5 µl 10x amplitaq gold buffer, 2.5 mM MgCl₂, 0.2 mM deoxyxynucleotide triphosphate, 0.9 units of AmpliTaq Gold (Perkin-Elmer, Wellesley, MA, USA), 1 µl DNA, 0.05 µCi [³²P]dATP, and 50
forward and 30 ng reverse primer. Five-minute denaturation at 95°C was followed by 35 cycles of 30 sec at 95°C, 45 sec at the appropriate annealing temperature (Table I), and 45 sec at 72°C. Elongation was achieved by 10 min at 72°C followed by chilling to 4°C. The PCR products were mixed with 13 μl of loading buffer (95% formamide, 20 mM EDTA, 0.05% bromphenol blue and 0.05% xylene cyanol), denatured for 5 min at 95°C, and kept on ice. Then 4 μl of the PCR product was loaded on a denaturing 6% polyacrylamide gel containing 7 M urea and run at 65 W for 1.5–2 h. The gels were dried and radiographed. Autoradiograms were evaluated by visual inspection. Allelic imbalance was defined as near or complete loss of a band in the tumour relative to the corresponding normal sample. Allelic conservation was defined as the clear presence of both alleles in both abnormal and corresponding normal DNA. All of the other situations were judged as non-informative.

Fisher’s Exact Test was used for comparisons between individual polymorphic markers in the two tumour groups. The Mann-Whitney U-test was used for comparison of the total number of allelic loss. $P = 0.05$ (two-sided) was taken as the limit of significance. A $p$-value between 0.05 and 0.10 was considered a statistical trend.

Table I. Polymorphic markers 7q36.1-q36.3.

<table>
<thead>
<tr>
<th>Amplicon</th>
<th>Location</th>
<th>$T_{an}$</th>
<th>Size</th>
<th>Forward primer</th>
<th>Reverse primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>D7S636</td>
<td>150137074-150137247 band 7q36.1</td>
<td>55</td>
<td>173</td>
<td>gag gag cta cta att gg</td>
<td>agc tgt tgt ggg gtt cag</td>
</tr>
<tr>
<td>D7S2439</td>
<td>150576614-150576769 band 7q36.1</td>
<td>57</td>
<td>155</td>
<td>cag cta aag gta cag caa ttt c</td>
<td>gta taa cat agg ttc atc ggc</td>
</tr>
<tr>
<td>D7S483</td>
<td>151635828-151635960 band 7q36.1</td>
<td>57</td>
<td>132</td>
<td>tca tta ggc tgg gaa tta a</td>
<td>ttc tct tct gct gtc ace aaa</td>
</tr>
<tr>
<td>D7S550</td>
<td>155017029-155017183 band 7q36.3</td>
<td>56</td>
<td>154</td>
<td>cat cca caa tcc act cct ag</td>
<td>gat gtt gtg att aga gtt gct g</td>
</tr>
<tr>
<td>D7S3037</td>
<td>155252970-155253119 band 7q36.3</td>
<td>58</td>
<td>149</td>
<td>cag ctc agg gca gga ttc ta</td>
<td>aag gag tca tct gac age aa</td>
</tr>
</tbody>
</table>

1 Location and size according to the UCSC Genome Browser on the Human May 2004 Assembly.
2 $T_{an}$ = Annealing temperature used in PCR reaction.
3 Size of the amplicon in base pair.

A: Oesophageal (Barrett’s) adenocarcinoma

B: Gastric cardia adenocarcinoma

Figure 1. Overview of allelic imbalances in adenocarcinomas of (A) Barrett’s oesophagus and, (B) gastric cardia. Black boxes represent allelic imbalance; white boxes, conservation; grey boxes, not informative.
Results

The 7q36.1-q36.3 region was examined with a set of 5 polymorphic markers in 33 Barrett-related carcinomas. Moreover, 40 gastric cardia cancers were investigated to compare the pattern of imbalance at these loci. The results obtained by LOH analysis are summarized in Figure 1, examples of LOH are shown in Figure 2. Overall, the number of allelic loss was more frequent in Barrett’s cancers than in gastric cardia carcinomas (p=0.04). The spectrum of imbalance was found to differ between the two cancer sites (Figure 3). In Barrett’s adenocarcinomas, allelic loss varied from 28% to 45% (of informative cases) with the highest frequency at marker D7S483. In gastric cardia cancers, imbalance ranged from 12% to 27% (of informative cases), being most prevalent for marker D7S3037. The difference between the two cancers was highest for polymorphic marker D7S483 (p=0.05).

Discussion

In the literature there are few data concerning LOH of distal 7q. This region does not seem to be frequently lost in human cancers. Allelic imbalance of 7q35-q36 has been reported in leukaemia (10, 11). Recently, a breakpoint on 7q36 involving the ETV6 gene in infant leukaemia was found (12). Loss on 7q32.3-q36.1 has also been observed in gallbladder and oropharyngeal epithelial tumours (13, 14). In our study, the peak of loss was detected for marker D7S483 on 7q36.1 in Barrett’s adenocarcinomas, which also represented the genomic locus with the largest difference between oesophageal and gastric cardia cancers. A clear peak of loss was not discerned for gastric cardia carcinomas. Two possibly important genes within our critical region between markers D7S483 and D7S550 are XRCC2 (151735329-151764991 bp; band 7q36.1; ref.15) and DPP6 (153394143-154076902 bp; band 7q36.2; ref.16). XRCC2 is involved in double strand DNA repair, which might explain the profound genomic instability seen in oesophageal and gastric cardia adenocarcinomas. DPP6 is involved in post-translational modification of proteins and it might play an important "check-point" role in carcinogenesis.

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References


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