SHORT COMMUNICATION

Human connexin 37 is polymorphic but not mutated in tumours

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Connexins are phylogenetically conserved proteins responsible for gap junctional intercellular communication (GJIC). In tumours, GJIC is frequently disrupted. We have tested the hypothesis that the connexin 37 (Cx37) gene might be mutated in human tumours from tissues in which the Cx37 gene is known to be expressed. Eight lung adenocarcinomas and 18 sporadic breast carcinomas were analysed. While most tumours had GTA at codon 130, a base change GTA—ATA converting valine into isoleucine was found in three breast cancers (one homozygous for ATA) and two lung tumour samples. However, screening of normal DNA from the same patients and DNA from 42 healthy donors revealed that such base change also exists in normal tissue. Thus, we conclude that there is polymorphism of the connexin 37 gene in the human population. This is the first finding of polymorphism in the connexin gene family.

Gap junctional intercellular communication (GJIC*) plays an important role in maintenance of tissue integrity, by transferring messengers of low molecular weight between cells (1,2). GJIC is thus considered to be critically involved in such functions as tissue homeostasis, growth control, tissue response to hormones and regulation of embryonic development. Dysfunction of GJIC has been found in a number of pathological conditions, including cancers. The role of GJIC impairment in carcinogenesis is well documented (3,4). Lack of homologous and/or heterologous GJIC has been found in almost all tumour cells and supports the idea that defective GJIC creates conditions for accelerated clonal uncontrolled expansion and progression of tumour cells. In addition to extensive data from in vitro studies, our results obtained with an experimental rat model and human liver tumours clearly demonstrate a major deficiency of GJIC function in tumour cells and communicational isolation of tumours from surrounding non-tumourous tissue in vivo (5,6).

When connexin genes were transfected into tumour cell lines, the in vitro growth capacity and often tumourigenicity was reduced (7–9), suggesting a tumour-suppressing role of connexin genes. The strongest tumour suppressive effect was observed if a connexin gene, which is expressed endogenously in the original tissue, was transferred into tumour cells (10). We hypothesized that connexin genes may be mutated in primary human tumours, like many other established tumour-suppressor genes.

Connexin 37 (Cx37) is expressed in various organs including lung and breast (11–13). We decided to study Cx37 gene mutation in human lung and breast tumours. We examined DNA samples from eight lung tumours (five adenocarcinomas, three squamous cell carcinomas and one giant cell carcinoma) from Moscow, Russia (provided by Dr I.B.Zborovskaya, Russian Cancer Research Center) and 18 sporadic breast adenocarcinomas from Lyon, France (provided by Dr O.M.Serova, IARc). All of them were matched with internal control DNA from leucocytes. The tumour connexin 37 gene was analysed by direct sequencing of a polymerase chain reaction (PCR) product. The DNA sequence from the 206th (1st base of the 48th codon) to the 1083rd base (20 bases downstream from the 3’ end of the coding region) of the published sequence of Cx37 (Reed et al., 1993), which corresponds to 86% of the open reading frame, was amplified by PCR. These PCR products were used for a second PCR before direct sequencing. We used four pairs of primers giving overlapping fragments in order to read the maximum of the connexin 37 sequence (Table I).

As shown in Figure 1, we found different base sequences in codon 130. Most tumours had G in both alleles in the first position of codon 130, but one of the 26 tumour samples had A in both alleles (Table II). Thus, codon 130 coded for valine in 21 tumours, both valine and isoleucine in four tumours and for isoleucine in one. In addition, we found that several nucleotides differed from the published sequence (12). The differences between our results and the published sequence were found in codons 112 (GAG—GGG), 129 (GGC—GCC), 132 (CTT—CGT), 147 (ATT—ATC), 148 (CCG—CGC) and 149 (CGA—GGA). Since our sequence was found in all the samples we studied, we believe that the published sequence is not accurate.

In order to see whether these Cx37 sequence variations at codon 130 represent somatic mutations in tumours or rather genetic polymorphisms, we analysed DNAs of normal leucocytes from the same patients. These DNAs had the same base sequence as normal tissue.

Fig. 1. Polymorphism at the 130th codon of the Cx37 gene. PCR fragment corresponding to the 98th to 181st codons of Cx37 was directly sequenced. Arrows indicate positions of the first nucleotide of the codon 130. A, homozygous for G; B, heterozygous; C, homozygous for A.

Abbreviations: Cx37, connexin 37; Cx32, connexin 32; GJIC, gap junctional intercellular communication; PCR, polymerase chain reaction.
We have found no mutations of the connexin gene 32 (Cx32) gene in human primary tumours of the liver or stomach (6,15). In one rat liver tumour induced by a nitrosamine, we found a base-substitution mutation in the Cx32 gene (16), but cloning and expression experiments indicated that no functional change was associated with this mutation (Omori et al., in preparation).

While connexin gene mutations are infrequently found in human tumours, at least two types of human genetic disease are associated with germ-line mutations of connexin genes. X-linked Charcot–Marie–Tooth syndrome has been reported to be due to Cx32 gene mutations (17–20) and some types of heterotaxia are associated with Cx43 gene mutations (21). Cells containing some of these mutations have been confirmed to show aberrant GJIC or none at all (18). In addition, mutant Cx32 proteins appear to down-regulate wild type Cx32 proteins in a dominant-negative fashion by producing non-functional connexons composed of mutant and wild type Cx32 (18,22). These results suggest that a mutation of a connexin gene may result in a severe biological disorder.

We have shown that there is Cx37 gene polymorphism in the human population. This polymorphism results in an amino-acid change at codon 130 (valine and isoleucine). This amino acid is situated in the cytoplasmic loop of the Cx37 molecule, an area considered to be less conserved than transmembrane and extracellular domains among various connexin species, but highly conserved in various organisms. Whether and to what extent such a polymorphism results in functional changes of GJIC is not known. Our results, although based on only small numbers of samples, indicate that the polymorphism is not associated with a higher or lower prevalence of lung or breast cancers, suggesting no direct link between the polymorphism and malignant transformation in these two organs. On the other hand, a recent study on another connexin, Cx43, has shown that the cytoplasmic loop plays an important role in GJIC (23). Since the Cx37 polymorphism we found in this study is located at the cytoplasmic loop, it is possible that this polymorphism is associated with other human diseases.

Acknowledgements
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Table I. Sequence of oligonucleotide primers used

<table>
<thead>
<tr>
<th>Primer</th>
<th>Primer location</th>
<th>Sequence</th>
<th>Size (bp) of predicted product</th>
</tr>
</thead>
<tbody>
<tr>
<td>First PCR</td>
<td>Forward</td>
<td>206–225</td>
<td>GAGCAGTCAGATTTCCAGAGTG</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>1064–1083</td>
<td>ACATAAAGCCACAGCGCTCTA</td>
</tr>
<tr>
<td>Nested primers used in second PCR</td>
<td>Forward</td>
<td>358–376</td>
<td>CCGTGCTCCGCGGAGAAAGAG</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>582–601</td>
<td>CCAGCCGTACAGCGCCACT</td>
</tr>
<tr>
<td></td>
<td>Forward</td>
<td>766–785</td>
<td>TCACTCCCCCCCCTGAGCCAG</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>739–758</td>
<td>GCTGGAGTTTGCGACCTG</td>
</tr>
<tr>
<td></td>
<td>Forward</td>
<td>876–895</td>
<td>TCTTCTCTACGCCCCCTC</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>853–872</td>
<td>TCTTCTCTACGCCCCCTC</td>
</tr>
<tr>
<td></td>
<td>Forward</td>
<td>1064–1083</td>
<td>ACATAAAGCCACAGCGCTCTA</td>
</tr>
</tbody>
</table>

Table II. Cx37 gene polymorphism at the first nucleotide of the 130th codon in patients bearing tumours and in healthy donors

<table>
<thead>
<tr>
<th></th>
<th>G/G (%)</th>
<th>G/A (%)</th>
<th>A/A (%)</th>
</tr>
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<tbody>
<tr>
<td>Cancer patients (n = 26)</td>
<td>21 (80.9)</td>
<td>4 (15.3)</td>
<td>1 (3.8)</td>
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<tr>
<td>Breast adenocarcinoma (n = 18/26)</td>
<td>15</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Lung tumour (n = 8/26)</td>
<td>6</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Lymphocytes from healthy donors (n = 42)</td>
<td>33 (78.5)</td>
<td>6 (14.3)</td>
<td>3 (7.1)</td>
</tr>
</tbody>
</table>

*In all cancer patients, the same genotype was found in tumours and their lymphocytes.
References


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