Short Communication

Somatic glypican 3 (GPC3) mutations in Wilms’ tumour

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Tumour and normal tissue from 41 male cases of Wilms’ tumour were screened to determine the presence of sequence variants in the glypican 3 (GPC3) gene. Two non-conservative single base changes were present in tumour tissue only. These findings imply a possible role for GPC3 in Wilms’ tumour development.

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It has been known for many years that the risk of Wilms’ tumour (WT) is greatly increased in children with certain congenital abnormalities and syndromes, but the complexity of WT genetics has only been recognised more recently (Hastie, 1994). Thus far the only WT gene to be fully characterised is WT1, located on chromosome 11p13 but deletion or mutation to WT1 is found in only about 20% of sporadic WT (Huff, 1998). Beckwith-Wiedemann syndrome (BWS) is characterised by pre-and/or post-natal overgrowth and a variety of other congenital abnormalities and confers a greatly increased risk of WT (DeBaun and Tucker, 1998). Other overgrowth syndromes may also predispose to WT. Numerically the most important of these is Simpson-Golabi-Behmel syndrome (SGBS) which shows an X-linked pattern of inheritance. WT has been noted in a number of patients with SGBS (Xuan et al, 1994, 1999; Hughes-Benzie et al, 1996; Lindsay et al, 1997).

Constitutional deletions or mutations in the glypican 3 gene (GPC3) at Xq26 are found in many SGBS families (Pilia et al, 1996). This suggests that deletion or mutation in GPC3 may be involved in the development of some WT cases. The possibility that somatic mutations to GPC3 may be present in sporadic WT not associated with SGBS therefore also arises. To investigate this possibility we have analysed an unselected series of WT cases, for the presence of somatic and constitutional alterations to GPC3 in tumour and normal tissue respectively.

MATERIALS AND METHODS

Approval for the project was given by the relevant research ethics committees. Histopathological material from incident cases of WT included in the Manchester Children’s Tumour Registry (Blair and Birch, 1994) was examined. Paraffin blocks containing tumour tissue and normal kidney respectively, were selected for each case.

Paired tumour and normal tissue samples were available on 41 male cases. Seven different variants were detected. Variant 4 was present in three cases but the remaining six variants were detected in single cases only. Variants 1–5 were present in both tumour derived and the corresponding normal DNA. Variants 6 and 7 were detected in tumour derived material only (Figure 1).
Variants 1 and 3 were present in the same case. Variant 1 is due to a silent T – C transition in exon 7, position 1697 (all nucleotide numbering refers to the sequence described by Huber et al., 1997). Variant 3 is due to a silent A – G transition in exon 8 position 1823. Both variants were reported in three out of 13 ovarian cancer cell lines, and always occur together (Lin et al., 1999). Variant 2 is the result of a G – A transition in exon 5, position 1482, which changes non-polar valine to non-polar methionine. We have observed the same sequence variant in blood samples from two out of 36 unselected breast cancer patients (i.e. 72 X chromosomes). Variants 4 and 5 are the result of the reduction in a run of 16 Ts in the splice acceptor region of exon 3 – 15 and 14 respectively. We consider variants 1 – 5 to be polymorphisms with no predicted effect on splicing.

Sequencing showed variant 6 to be due to a C – T transition in exon 3 position 558. This changes a basic histidine to an uncharged polar tyrosine. Variant 7 is due to a G – A transition in exon 8, position 1902. This changes the non-polar alanine to the polar threonine. Variants 6 and 7 are tumour specific i.e. they were present in tumour DNA but were not detected in DNA from normal kidney and may represent somatic mutations.

DISCUSSION

GPC3 is one of six glypican genes which so far have been identified in vertebrates. Glypican 3, encoded by the GPC3 gene, is a GPI-linked heparan sulphate proteoglycan. It is highly expressed in embryonic mesodermal tissues corresponding to the tissues showing over-

Table 1 Glypican 3 (GPC3) oligonucleotides used in this study

| Exon | Primer | Sequence |
|------|--------|----------|
| 1    | 1B*    | 5'TAGGCACGCTCAAGGGAC' |
| 1    | 1F     | 5'TCCTACCTCCCTGGAGACA' |
| 2    | 2A*    | 5'TGTTCGGCTTGTTCCGATG' |
| 3    | 3A*    | 5'SGAATATCGGCACTAAGC' |
| 3    | 3B*    | 5'SCTGCTCTGGTTCCGAC' |
| 3    | 3C*    | 5'SCTGCTCTGGTTCCGAC' |
| 3    | 3D*    | 5'STTGAAAGACAGGACACG' |
| 3    | 3E*    | 5'GAACGAAAATCATCTGTTCC' |
| 3    | 3F*    | 5'TACTGCTGACAGTGTAGAC' |
| 3    | 3G    | 5'SCTGGAATCATGAAGGACTG' |
| 3    | 3H    | 5'ACCATGATCGTCCCCGCA' |
| 3    | 3I    | 5'ATGTGAGAGGACGACATCTGTA' |
| 3    | 3J    | 5'CAAGCTCTGACTCCCAAAGC' |
| 4    | 4A*    | 5'CTTATCATCTGACGACAGTATC' |
| 4    | 4B    | 5'GAATTTGTTATTGATTTGAGGAT' |
| 5    | 5A*    | 5'GCCCCTCTGAGACATG' |
| 5    | 5B    | 5'GCTTAAATGCTAATGACAAC' |
| 6    | 6A*    | 5'TGAACTGATGCTACCAAGTGA' |
| 6    | 6B    | 5'TCCCTCTCTCGTTATCTCTTCAC' |
| 7    | 7A*    | 5'GAGAGCTGATGCAATCC' |
| 7    | 7B    | 5'GATTGAGCTGACAGCATACTGAC' |
| 8    | 8A*    | 5'GTTTATATCATGGCTGTAG' |
| 8    | 8B    | 5'GTTTATATCATGGCTGTAG' |

Some denoted as * have been described previously (Huber et al., 1997). More detailed sequence information is available with GenBank accession numbers AF003529, Z99570, AL009174, AL008712 and AC002420.

Table 2 Details of the main primer pairs and PCR conditions used to provide total coverage of the coding region of GPC3 for SCCP analysis

| Exon | Primer pair | Buffer | DMSO (±5%) | Annealing temp (°C) | Product size (bp) |
|------|-------------|--------|------------|--------------------|------------------|
| 1    | 1B/1F       | Roche  | +          | 60                 | 225              |
| 2    | 2A/2B       | Roche  |           | 60                 | 329              |
| 3    | 3A/3J       | Roche  | –          | 59                 | 183              |
| 3    | 3K/3B       | Roche  | +          | 60                 | 211              |
| 3    | 3C/3H       | TNK100 |           | 55                 | 255              |
| 3    | 3G/3D       | Roche  | –          | 58                 | 243              |
| 3    | 3E/3F       | Roche  | –          | 55                 | 173              |
| 4    | 4C/4D       | TNK100 |           | 57                 | 231              |
| 5    | 5A/5D       | Roche  | –          | 57                 | 200              |
| 6    | 6C/6D       | Roche  | –          | 60                 | 279              |
| 7    | 7A/7C       | Roche  | +          | 59                 | 221              |
| 8    | 8A/8B       | Roche  | –          | 60                 | 296              |
growth or other abnormalities in SGBS (Selleck, 2000; De Cat and David, 2001). It is the only glypican to date for which mutations in humans have been documented.

We have detected somatic point mutations in the GPC3 gene in two out of 41 WT cases (4.9%). This represents the first fully documented report of such mutations in tumour tissue and provides evidence that disruption of the GPC3 protein may be involved in initiation, development or progression in some Wilms’ tumours. Support for such a role is provided by the observation that GPC3 is expressed in WT tissue but not in the corresponding normal kidney (Saikali et al., 2000). The 4.9% GPC3 mutation frequency detected in the present series may be an underestimate since the methods used did not include analysis of the promoter region and would not have detected large deletions of GPC3 exons which occur in some SGBS patients (Hughes-Benzie et al., 1996; Pilia et al., 1996; Lindsay et al., 1997). Southern blotting is not suitable for the archival material available to us, but we have partially addressed this issue by carrying out multiplex PCR analysis of GPC3 exons and a control gene for 20 of the WT samples. Results (data not shown) showed no evidence for deletions.

The mutation in exon 8 occurs near to the C terminus in a predicted region of glycosylphosphatidylinositol (GPI) anchorage. A number of deletions detected in patients with SGBS also affect exon 8 and probably prevent attachment of any product to the cell membrane (Hughes-Benzie et al., 1996; Pilia et al., 1996; Lindsay et al., 1997). Furthermore, the GPI-anchoring domain has been identified as critical for the induction of apoptosis in mesothelioma and breast cancer cell lines (Gonzalez et al., 1998). The mutation in exon 3 does not appear to be in any known functional domain but is non-conservative and may be expected to affect protein conformation. The five published cases of WT in patients with SGBS in whom alterations in the GPC3 gene have been detected were all associated with constitutional deletions in exon 1 and/or exon 2 (Hughes-Benzie et al., 1996; Pilia et al., 1996; Lindsay et al., 1997). Additional studies are required to establish the functional consequences of these putative somatic mutations.

In conclusion in this preliminary study, we have identified somatic mutations in the GPC3 gene in two of 41 cases of WT, providing evidence of a link between developmental genes and embryonal tumours. Further investigations of the possible role of GPC3 in WT are indicated.

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