Studies on the pathogenesis of Costello syndrome

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Costello syndrome is characterised by high birth weight, early psychomotor and growth retardation, cardiomyopathy, relative macrocephaly, coarse face, and laxity of the small joints. Skin abnormalities include nasal and perianal papillomata, acanthosis nigricans, cutis laxa, and curly and sparse scalp hair.1 7

Increased paternal age and sporadic occurrence have suggested autosomal dominant de novo mutations.7

Recently, several solid tumours have been described in patients with Costello syndrome, such as bladder carcinoma (2), rhabdomyosarcomas (3), vestibular schwannoma (1), and epithelioma (1). The estimated frequency of tumours is about 17%. Whereas rhabdomyosarcomas and neuroblastomas are relatively frequent in childhood, our attention was drawn to bladder carcinoma because it is extremely rare in childhood. Its occurrence has been suggested to be specific for this syndrome.7

FGFR3 is the oncogene most often mutated in bladder cancer, with mutations shown in more than 40% of carcinomas.3 9 Activating mutations have been found in “hot spots” of exons 7, 10, and 1.5 Also FGFR3 somatic mutations are found in 3–25% of cervical carcinomas8 and 14% of multiple myelomas.11 12

FGFR3 dominant germline mutations can cause different types of chondrodysplasia (achondrodysplasia and hypochondrodysplasia, thanatophoric dysplasia, and SADDAN) or some craniosynostosis syndromes (reviewed by Vajo et al9). Among these, the variant of Crouzon syndrome and SADDAN are associated with acanthosis nigricans and hyperpigmentation which are also found in patients with Costello syndrome.

Recent biochemical studies showed that cutis laxa is caused by impaired assembly of elastin fibres, owing to a functional deficiency of the 67 kDa elastin binding protein. This protein is inactivated by abnormal binding to excessive mucopolysaccharides, which accumulate in cultured fibroblasts of patients with Costello syndrome.13 The observation of chondroitin sulphate storage in the lysosomal compartment suggests a defect in mucopolysaccharide degradation. Also a high rate of cellular proliferation in fibroblast cultures was found, suggesting a constitutive activation of the fibroblast growth factor pathway.

We studied the pathogenesis of this disorder in five patients by a molecular and a biochemical approach. Considering the heterogeneity of syndromes caused by FGFR3 germline mutations, we searched for FGFR3 gene mutations. Activation of the FGF signal transduction pathway also depends on extracellular heparan sulphate mucopolysaccharides. Consequently we studied mucopolysaccharide turnover by the sulphate incorporation test and by measuring the activity of a novel lysosomal sulfatase involved in heparan sulphate metabolism. Finally we checked whether the sialuria found earlier depends on an intracellular sialic acid abnormality, by measuring the sialic acid content of cultured fibroblasts.

PATIENTS AND MATERIALS

We collected skin fibroblasts from five unrelated patients with Costello syndrome. The first three were diagnosed at the Department of Paediatrics, University of Genova, Italy, and are here identified respectively as patients 1, 2, and 3. Patients 1 and 2 have been previously described,11 whereas patient 3 was later diagnosed by one of the authors (MDR) by similar clinical criteria and died in infancy. The sialic acid excretion of this patient was 424 µg/mg creatinine, with a 38% ratio free/total (age matched controls 261 µg/mg creatinine, with 33% ratio free/total) (fig 1).

Patient 4 was diagnosed at the Department of Paediatrics of the University of Napoli, Italy. She presented with neonatal asphyxia, severe postnatal failure to thrive, and moderate psychomotor retardation. On clinical examination the face was coarse with wide alae nasi, abnormal skin hyperpigmentation on the left arm, dystrophy, diffuse muscle atrophy, and hypotonia. She developed a progressive obstructive hypertrophic cardiomyopathy but, by the age of 9 years, the psychomotor and growth delay had improved. Malabsorption, perinatal infections, visceromegaly, and ocular defects were excluded. Her urinal sialic acid at the age of 8 months was 728 µg/mg creatinine, with a 38% ratio free/total. (All sialic acid determinations in urine were performed in the laboratory of Professor...
tion and genomic sequence analysis were performed for exons

We analysed DNA from patients 1, 2, 4, and 5 for mutations in the FGFR3 gene. Polymerase chain reaction (PCR) amplification and genomic sequence analysis were performed for exons 7, 10, 13, 15, and 19. These exons were chosen because all the mutations found so far were within them. They also covered the hot spots often mutated in bladder carcinoma. No pathogenic mutation was found, the only change found being a known N294N polymorphism in exon 7 in patient 4. The results do not support the hypothesis that frequent germline mutations in the FGFR3 gene are associated with Costello syndrome.

Because of the earlier report of accumulation of chondroitin sulphate in Costello fibroblasts, we tested glycosaminoglycan turnover by measurement of total sulphate incorporation in mucopolysaccharides, with radiolabelled NaSO₄ as previously described. This test is a good measure for mucopolysaccharide turnover in fibroblasts and is abnormal in most mucopolysaccharidoses.

Because of the interaction between cell surface heparan sulphate and FGF receptors, to test for a defect in the FGF pathway, we used this test to compare the pericellular (cell bound) sulphurylated mucopolysaccharides with the intracellular content.

[S]-NaSO₄ incorporation in cells from all five patients, expressed as patient/control ratio, was normal (0.8–1.6). No significant difference was found between the pericellular and the intracellular pool. In the same assay, fibroblasts from a patient with Hunter’s syndrome showed a ratio of 4.3 for the intracellular and 2.1 for the pericellular sulphurylated mucopolysaccharides.

Table 1 Glycan metabolism in Costello fibroblasts

<table>
<thead>
<tr>
<th>Patients</th>
<th>Free NeuA C (nmol/mg pr)</th>
<th>[³⁵S] Sulphate incorporation (ratio patient/control; pericellular pool vs. intracellular pool)</th>
<th>Glucuronate-2-sulphatase (nmol/mg/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.4</td>
<td>1.6 (1.1)</td>
<td>47</td>
</tr>
<tr>
<td>2</td>
<td>0.6</td>
<td>0.8 (1.5)</td>
<td>57</td>
</tr>
<tr>
<td>3</td>
<td>0.8</td>
<td>0.8 (1.2)</td>
<td>81</td>
</tr>
<tr>
<td>4</td>
<td>0.4</td>
<td>1.2 (ND)</td>
<td>85</td>
</tr>
<tr>
<td>5</td>
<td>0.5</td>
<td>1.2 (ND)</td>
<td>148</td>
</tr>
</tbody>
</table>

Free sialic acid content was measured according to Mancini et al. Sulphate incorporation was measured according to Fortuin and Kleijer. The results represent the ratio between patient and control cell associated radioactivity. Between brackets are the values of cell bound radioactivity obtained after trypsinisation of fibroblasts at the end of the incubation with [³⁵S]NaSO₄. Fibroblasts from a Hunter patient as internal control showed an abnormal ratio of 4.3 for intracellular and 2.1 for pericellular sulphurylated mucopolysaccharides.

Glucuronate-2-sulphatase was assayed with a novel 4-methylumbelliferyl substrate synthesised by an adaptation of the method of Voznyi et al. Glucuronate-2-sulphatase activity was measured with a novel 4-methylumbelliferyl substrate synthesised by an adaptation of the method of Voznyi et al.

DISCUSSION

We concluded that the somatic mutations associated with bladder carcinoma are not also associated with Costello syndrome. We also did not find mutations in the exons of the gene that have so far proved to be susceptible to both germline and somatic mutations, such as those causing chondrodysplasias, craniosynostosis, and solid tumours. It is still possible, although unlikely, that mutations are present in the areas of the gene, including the promoter area, which were not analysed. Furthermore, our results could be biased by the limited number of patients.
We found that free sialic acid is normal in Costello fibroblasts. The sialuria earlier reported in our patients is apparently not related to known genetic defects of intracellular sialic acid metabolism, such as non-lysosomal sialuria (MIM 269921) and sialic acid storage disease (MIM 604369, MIM 269920). Non-specific sialuria has been found in CDG syndrome and in childhood nephropathies. 20,21  

An intracellular accumulation of chondroitin non-sulphate, as a cause of functional deficiency of the 67 kDa elastin binding protein, has been described in fibroblasts of patients with Costello syndrome. 22 This gives support to the previous hypothesis of a defect in lysosomal degradation. 23 

The heparan sulphate proteoglycans associated with membranes are the most intensively studied proteoglycans involved in growth control. 24  

In fibroblasts from patients with Costello syndrome, 22 an unusually high rate of cellular proliferation was also found, as was an increased expression of CD44, a cell surface hyaluronate receptor, which provides a link to the hypothesis of a defect in the growth factor pathway. 

Heparan sulphate proteoglycans are necessary for the binding of FGF to the FGFR3b, namely, the receptor type that is expressed in epithelia and connective tissue. Therefore we quantitatively tested glycosaminoglycan metabolism by the sulfate incorporation test. This test gives a measure for mucopolysaccharide (including chondroitin, heparan, and derma- tan sulphate) turnover and detects intracellular and pericellular distribution. 25 We found no abnormality, even when we corrected for the pericellular pool. 

The heparan sulphate degrading glucuronate-2-sulphatase is a recently discovered lysosomal enzyme not yet linked to any metabolic disease. 26 We measured a normal activity of glucuronate-2-sulphatase in fibroblasts from patients with Costello syndrome. 

It is still possible that the chondroitin sulphate abnormality reported in fibroblasts from patients with Costello syndrome reflects a qualitative rather than a quantitative abnormality of mucopolysaccharide composition. Alternatively, the accumulation is below the detection limit for the assay used.

Both the phenotypic abnormalities and the tumorigenicity of Costello syndrome could be explained by our hypothesis of a defect in the FGF signal transduction pathway. 

It is still possible that FGFR3, if not directly, is indirectly involved in Costello syndrome through a putative defect in proteoglycan metabolism. 

Recently a patient with a chromosome 1;22 translocation and Costello syndrome has been found. In theory the locus for Costello syndrome could be at the breakpoints of one of these two chromosomes. 27 Genes involved in FGF signal transduction at these loci should be, in our opinion, among the functional candidates for this disease.

ACKNOWLEDGEMENTS

We thank Joke Keulemans, Victor Garritsen, and Arjenne Janssen for technical assistance with the biochemical tests and sequence analysis of FGFR3.

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