

Finally, the last issue raised by Dietz *et al.* is that of genetic heterogeneity. Our belief in genetic heterogeneity in MFS stems not only from the linkage data in our family, but also from published data showing that it has been impossible to identify mutations in the fibrillin gene in a number of patients and that in some individuals with 'definite Marfan syndrome' no anomaly was observed in fibrillin metabolism. Although it is likely that normal fibrillin metabolism does not exclude the presence of an abnormal fibrillin associated with perturbation of microfibrillar structure and function, microfibrils contain not only fibrillin but also other components. Therefore, it is also possible that these MFS patients have abnormal microfibrillar structure or function due to anomalies carried by another component or by proteins associated with microfibrils. This interpretation has never been seriously considered and no effort has been undertaken to investigate fully the question of genetic heterogeneity despite

knowledge, as early as 1991 (refs 16,17), that in the French family the mutation locus was not *MFS1* (fibrillin). Consequently, it is not surprising that in a family in which fibrillin haplotypes did not segregate in all affected members, heterogeneity was not evoked. The absence of cosegregation was explained by the simultaneous existence of two different connective-tissue disorders (MFS and another still unknown)¹⁸, a very unlikely situation except if one is willing to consider that connective-tissue disorders are frequent. More alarmingly, under the cover of homogeneity, haplotype analyses with fibrillin polymorphic markers were used for diagnosis in an at-risk individual, in a small family in which the presence of a fibrillin defect had not been conclusively demonstrated¹⁸.

Indeed, genetic heterogeneity for MFS is a major issue to which our results make a significant contribution and which now requires a large objective and open-minded collaborative effort, in the interest of patients and their families.

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Immune surveillance in colorectal carcinoma

Sir — The identification of DNA mismatch correction defects in both sporadic and hereditary (nonpolyposis) colorectal adenocarcinomas¹⁻³ has highlighted tumour cells with a spontaneous mutator phenotype. Mutator tumour cells are potentially prime targets for cytotoxic T cells because they will express many neoantigens derived from genetically altered proteins. This selective vulnerability to immune surveillance is of potential therapeutic value. However, surviving tumour cells with a mutator phenotype may have undergone a particularly severe selective pressure to escape T-cell cytotoxicity through loss of HLA expression.

All the colorectal car-

cinoma cell lines in which a mutator phenotype and/or mismatch repair defect has been identified and HLA expression has also been investigated are presented in Table 1. We have

identified three lines — LoVo, DLD1 and HCT15 — which have a mutator phenotype and are defective in mismatch binding in a band-shift assay. A fourth line, SW48, is also a mutator but has normal mismatch binding⁴. LoVo, DLD1 and HCT15 were known to exhibit microsatellite instability⁵ and we have established that SW48 shares this phenotype. Remarkably, the four mutator lines were the only ones among 37 colorectal carcinoma cell lines analysed previously which failed to express surface β 2-microglobulin (β 2M)^{6,7}. β 2M is absolutely required for antigen presentation for HLA class I-mediated cytotoxic T cell responses. Three other lines — LS174T (ref. 5), HCA7 and

Table 1 β 2 microglobulin/HLA expression in mutator colorectal adenocarcinoma cell lines

Cell line	Microsatellite instability	Mutator phenotype	β 2M expression ^a
LoVo ^b	++ ⁵	++ ⁴	-/-
DLD1/HCT15 ^b	++ ⁵	++ ⁴	-/-
SW48 ^c	++ ⁴	++ ⁴	-/-
LS174T	++ ⁵	ND	+/+ ^d
LS411	++	ND	+/+ ^d
HCA7	++	ND	+/+ ^d
SW620	— ⁵	— ⁴	+/+
HT29	— ⁵	— ⁴	+/+
SW403	— ⁵	ND	+/+

+/+, homozygous WT.

-/-, homozygous or compound heterozygous for β 2M mutations.

^a β 2M expression determined by ELISA assay⁷.

^bDLD1 and HCT15 were established from the same tumour. DLD1, HCT15 and LoVo are all defective in mismatch binding⁴.

^cSW48 has normal mismatch binding and has a mutator phenotype at microsatellite sequences and at the *HPRT* locus⁴.

^dLS174T and LS411 do not express surface HLA-A2. HCA7 does not express surface HLA-A1 (ref. 6).

ND, Not determined at *HPRT*. Established at microsatellites only.

LS411 (S. Cottrell & W. F.B., unpublished results) — also display the microsatellite instability indicative of a mutator phenotype. Previous work⁶ has established that LS174T and LS411 are both genotypically HLA-A2 but neither expresses this antigen on the cell surface. Similarly, HCA7 cells are genotypically HLA-A1 but fail to express surface HLA-A1 molecules. Eight other lines that do not exhibit microsatellite instability, and are therefore unlikely to have a mutator phenotype, show no loss of surface HLA expression⁶ (S. Cottrell & W.F.B., unpublished results). This extensive correlation between HLA loss and a mutator phenotype, which was previously inferred on

the basis of very preliminary data⁷, provides compelling circumstantial evidence that mutator tumour cells have undergone selection to evade T-cell surveillance.

From a practical standpoint, the correlation between mutator phenotype and HLA loss suggests that immunotherapy against colorectal carcinoma may be of limited value. The emergence of a mutator phenotype is an early event in carcinoma development⁸ and selection against T-cell immunity may have done its irreversible work by the time the carcinoma is established. The challenge here is to design therapies aimed specifically at cells with a mutator phenotype that have lost surface HLA expression.

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Effect on splicing of a silent FGFR2 mutation in Crouzon syndrome

Sir—Fibroblast growth factor receptor 2 (FGFR2) is a single membrane spanning tyrosine kinase with alternatively spliced variants. Mutations in FGFR2 have been found in Crouzon syndrome^{1,2} and other craniosynostotic conditions³. A re-

current mutation is a G to A transition that causes a synonymous change at a CpG doublet in the alanine codon at position 344 in exon B or the immunoglobulin-like (Ig) IIIc domain. It was reported previously in three patients^{1,2} and subsequently, we

detected one more case for a total of four in 50 (8%) Crouzon syndrome patients. Reardon *et al.*¹ speculated that this change would create an acceptor splice site. We suggested that this mutation may affect splicing of the gene, creating either an acceptor or a

Fig. 1 a, Diagrams of the normal, proposed acceptor and new donor FGFR2 splicing events are shown. The sequence of the ASO probes for the normal and the mutant cDNA product of the acceptor (spanning 5 bp at the 3' end of exon U, the new acceptor splice site, and 13 bp in exon B) and donor (spanning 7 bp 3' of the new donor splice site, including the splice site and extending 11 bp into exon D) splice sites are underlined. The asterisks represent the site of the G→A point mutation. b, The FGFR2 RT-PCR products were generated using primer 5'-CTATGCAGTAAATGGCTATCTC-3' in exon D for reverse transcription and with forward primer 5'-TACCGTAACCA-TGGTCAGCT-3' in exon 1 for PCR. A second PCR was performed with the first PCR product and nested primer pairs (forward primer 5'-CCCTACCTCAAG-GTTCTCAA-3' in exon U and reverse primer 5'-GGGAAGCTGTAATCTCCTT-3' in exon D). The products were separated by electrophoresis into a 2% agarose gel and stained with ethidium bromide. In the patient, the normal product (208 bp) and the mutant product (157 bp) are approximately the same intensity whether from lymphoblasts (shown in lane 2) or fibroblasts (not shown). Only the normal PCR product is present from normal fibroblasts (lane 1) and normal lymphoblasts (lane 3 and 4). *Phi*X174 RF DNA/*Hae*III marker is in lane M. Sequential ASO hybridization to the same dot blot was performed with a probe from the normal FGFR2 sequence and from oligonucleotides specific to cDNA products predicted for the new acceptor or donor site splicing (see Fig. 1a). Hybridization is detected with the control probe for all samples, although no hybridization is detected with the mutant acceptor splice site ASO. Hybridization to the mutant donor splice site ASO is detected only for the patient's RT-PCR products dotted in lane 2 and not with normal controls. The affected patient used in this study was from the FCMW Crouzon family previously described².

