the measurement of oestrogen receptor values in breast cancer biopsies between different laboratories. The article particularly cautions on the various conformational changes that may occur in the receptor and subsequently influence their recognition by the enyzme immunoassay kit distributed by the Abbott Laboratories (Chicago, Illinois).

I would like to bring to the attention of your readers a few additional variables that we found to critically influence the enzyme immunoassay in a study that was published by us earlier [2]. Our study [2] concurs with the study of Romain and associates [1] with regard to the conformational changes of the oestrogen receptor and the ability to be recognised by the antibody. We also found that unliganded receptor was recognised differently by the antibody assay than either the oestradiol or the diethylstilbestrol bound receptor. Therefore, biopsies of patients exposed to different levels of circulating hormones or other chemicals that recognise oestrogen receptor could give 'false values' by the enzyme immunoassay.

An important observation was made by us regarding the conformation of the receptor that was recognised by the enzyme immunoassay kit. It was demonstrated [2] that only the non-proteolysed 4S form of the receptor (i.e. the dissociated 8S form), and not the 8S form of the receptor, was recognised by the antibody in the kit. Sample dilution buffer provided in the enzyme immunoassay kit contains components (KCl) to cause such a dissociation [2]. Therefore, in-house buffers that prevent the 8S→4S conversion would prevent the recognition of the oestrogen receptor with the antibody. In addition, we found that the proteolysed form of the receptor that retained the ligand binding site for oestradiol was also not recognised by the kit. Since breast tumours contain varying degrees of proteases, some of which may cause an 8S→4S proteolytic conversion, the use of protease inhibitors should also be considered in quality control trials.

Romain and colleagues [1] have raised an important issue related to the need for quality control of enzyme immunoassay measurements of oestrogen receptor in different laboratories. Every effort should be made to minimise the inter- and intra-laboratory measurements of the oestrogen receptor and to maximise the conditions for the most favourable conformational form of the oestrogen receptor to be recognised by the enzyme immunoassay kit. Therefore, it is essential for there to be a consensus with regard to the buffers that are to be utilised with the enzyme immunoassay kit in the measurement of oestrogen receptor for clinical use.

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Mutational Analysis of the *MCC* Gene by Single-strand Conformational Polymorphism Analysis

K.J. Cripps, L.J. Curtis and A.H. Wyllie

A REGION on chromosome 5q21 is commonly deleted in sporadic colorectal carcinomas, and has been shown to contain the gene responsible for familial adenomatous polyposis (FAP), an inherited form of colorectal cancer [1, 2]. Several genes have been mapped to this region including both *APC* and *MCC* [3–6]. *APC* has since been shown to be the gene responsible for FPC, and is also mutated somatically in the majority of sporadic colon cancers. The role of *MCC* is, however, still uncertain. No mutations of *MCC* have been found in FAP patients, and only six somatic mutations in sporadic tumours have been reported [6, 7].

This study screened the 17 exons of the coding region of the *MCC* gene for mutations in a small subset of clinically important colorectal tumours from 5 non-polyposis patients who presented with the disease at a relatively young age (Table 1). We considered it possible that defects in *MCC* might be important in colorectal tumorigenesis in this subset of younger non-polyposis patients whilst having little input on the development of sporadic tumours in older patients. Mutational analysis was performed by the technique of single-strand conformational polymorphism analysis after amplification by the polymerase chain reaction (PCR-SSCP) on normal and tumour tissue as described previously [6] using PCR primers previously published [8]. We used DNA from these tumours previously grown as xenografts in SCID mice to eliminate the presence of any normal stromal elements which may have rendered mutational analysis difficult.

The method detected abnormalities in all 5 previously reported cases with *MCC* mutation. No mutations, however, were found in the coding region of *MCC* in any of the 5 young patients, although several polymorphisms were noted. A deletion polymorphism in exon 10 and a C-T conservative base change at codon 708 of exon 15 have been reported previously [9]. A novel polymorphism was observed in 2 of the 5 patients. Sequencing of these samples revealed a two base pair change (GC-TG) within intron 13 immediately prior to exon 14. This polymorphism appeared to be linked to the polymorphism in exon 15 and this was confirmed by extending the study of these two exons to an additional 19 constitutional DNA samples from young non-polyposis colorectal cancer patients and 18 normal control patients. The allele frequency of the novel polymorphism (B1 in Table 1) was 83% compared to the published sequence of

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Table 1. Clinico-pathological and molecular genetic data

Patient number	Sex and age (years)	Family history of colorectal disease	Location of tumour	Dukes stage	5q21 status*	*MCC* exon 10	*MCC* exon 14	*MCC* exon 15	*TP53* ICC	17p status†	*TP53* mutation	*Ki-ras* (codon 12)‡
1	Male 48	—	Ascending colon	C	Loss	A1/A1¶	B1/B1	C1/C1	+	Loss	cd248	Mutated
2	Male 40	—	Sigmoid colon	C	Loss	A2/A2	B2/B2	C2/C2	–	Non-informative	cd175	Wild-type
3	Male 45	Brother	Sigmoid colon	C	Retained	A1/A2	B1/B1	C1/C1	+	Retained	cd248	Wild-type
4	Male 39	Maternal aunt	Sigmoid colon	A	Loss	A1/A1	B1/B1	C1/C1	–	Non-informative	nd	Wild-type
5	Male 36	Father, two brothers, grandmother and aunt	Unknown§	D	Retained‖	A1/A1	B1/B1	C1/C1	–	Retained	nd	nd

* Previously identified by a variety of polymorphic probes flanking *APC* and *MCC*.
† Established from LOH at YNZ.22 or polymorphic loci within *TP53*.
‡ Established by sequencing.
§ Peritoneal carcinomatosis at operation.
‖ Retained at the one site informative within the *APC* gene.
¶ A1/B1/C1 refers to the more frequent allele in the population, whilst A2/B2/C2 refers to the less frequent polymorphic allele.
nd, not done.

the gene. No difference in frequency was observed between the normal and young colon cancer populations.

A recent study from this laboratory showed that whilst allele loss on 5q21 invariably involved both *MCC* and *APC*, no mutations of *MCC* could be detected by PCR-SSCP in the remaining copy of the gene [10]. These combined studies suggest that *MCC* does not have a role as an independent tumour suppressor gene in colorectal cancer, at least at the level studied.

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