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MSH2 Sequence Variations and Inherited Colorectal Cancer Susceptibility

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THE IDENTIFICATION of germline mutations causing colorectal cancer susceptibility is important for understanding the genetic pathogenesis of this common cancer and may enhance the management of affected patients and relatives. Therefore, we read with interest the article of Hall and associates [1] concerning the significance of the intronic splice acceptor site variation in the hereditary non-polyposis colon cancer (HNPCC) gene *MSH2*. Like Hall and colleagues [1], we have investigated the possible association between colorectal cancer and the T→C substitution at position 6 of the 5′ exon 13 splice site. Our results lend further support to the conclusion of Hall and colleagues [1] that this sequence variation represents a polymorphism rather than a mutation. Although we initially detected this change by single-strand conformational polymorphism (SSCP) analysis of *MSH2* in a chromosome 2-linked HNPCC family [2], the sequence variation did not segregate with colon cancer in the family. Furthermore, using a *XmnI* restriction site assay, we found no significant difference between the heterozygote frequency of this variant in 30 HNPCC probands (1/30) and normal controls (2/16).

Nevertheless, sequence variants in *MSH2* remain candidates for low penetrance cancer susceptibility mutations, and we have investigated a further variant for a possible association with familial or early onset colorectal cancer. We initially detected a GGC→GAC substitution (glycine→aspartic acid) at codon 322 in exon 6 of *MSH2* by SSCP analysis, in an individual with multiple primaries including colorectal cancer at age 36 years, and endometrial and ovarian cancers at age 45 years. The glycine at codon 322 is highly conserved and is present in yeast and human *MSH2* homologues [3, 4]. To investigate whether this change was likely to be a polymorphism or a pathogenic mutation, we designed a *HinfI* restriction site assay to specifically detect this variant and screened (i) 45 patients with early onset colorectal cancer (age <45 years); (ii) 50 patients with late onset colorectal cancer (>45 years); and (iii) 30 probands from HNPCC families and 72 normal controls. A further isolated patient with early onset colorectal cancer (age 19 years), whose parents are consanguineous, was found to be homozygous for G322D. Although none of the late onset patients or the HNPCC probands were positive for G322D, one of the 72 normal controls was heterozygous for this variant. This individual had no personal history of cancer at age 84 years, but further investigation of her family history revealed that two of her siblings were reported to have colorectal cancer at ages 41 and 80 years, respectively. However, we have not been able to establish whether either of these relatives had the G322D variant. Thus, although the G322D variant may represent a rare polymorphism, it is also a candidate for a low penetrance mutation or a modifying effect in patients with an inherited susceptibility to colorectal cancer. We hope that this report will encourage other groups to investigate the significance of this sequence variation.

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Chemotherapy with Doxorubicin, Etoposide and Cyclophosphamide (DEC) in Ovarian Cancer Persistent after Platinum-based Treatment

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SECOND-LINE treatments for persistent ovarian cancers provide discouraging results and investigation of new cytotoxic drugs

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Table 1. Response to DEC regimen in relation to the response at first-line treatment

Patients category	Tumour	CR	PR	NC	NEP	PD	NE
Potentially platinum sensitive	measurable	3	1	3	—	5	2
	unmeasurable	—	—	5	12	2	—
Platinum refractory	measurable	1	1	8	—	10	—
	unmeasurable	—	1*	—	1	—	—

CR, complete response; PR, partial response; NC, no change; NEP, no evidence of progression; PD, progression of disease; NE, not evaluable.
* Confirmed by laparoscopy.

and regimens is needed [1, 2]. The most significant factor in predicting response [3] is the interval from the end of previous treatment. To allow objective comparison of different trials, Markman and Hoskins [4] have recommended the classification of tumours in relation to prior platinum-based therapy: platinum resistant and potentially platinum sensitive tumours.

Between 1987 and 1989, 55 patients with persistent, advanced ovarian cancer were treated with the doxorubicin + etoposide and cyclophosphamide (DEC) regimen following adequate first-line chemotherapy with platinum compounds. 33 had potentially platinum sensitive and 22 had platinum resistant tumours. 34 subjects had evaluable disease whereas 21 had unmeasurable tumour detected at second-look surgery. Chemotherapy consisted of doxorubicin 40 mg/m^2 intravenous (i.v.) (day 1), cyclophosphamide 500 mg/m^2 i.v. (day 1) and etoposide 100 mg/m^2 i.v. (days 1, 2, 3). The cycles were repeated every 3 weeks. The patients received 1–14 courses (median 5 courses) and 53 subjects were evaluable for response. One patient discontinued the treatment after the first course because of grade 4 myelotoxicity whereas another suffered a cerebrovascular accident.

Among 32 evaluable patients with measurable tumour, 15 had progression of disease, 11 had stable disease and 6 had objective response (4 complete). We observed 2 (10%) responses in 20 primary platinum-resistant tumours and 4 (33%) in 12 potential platinum sensitive tumours.

Among patients without clinically evaluable disease, 5 had stable disease confirmed at laparoscopy, 1 had confirmed partial response. 13 had no evidence of progression and 2 had clinical progression. Table 1 summarises the response to treatment in relation to response at first-line treatment.

Four years after the enrolment of the last patient, 50 subjects have died of disease, 1 of bone marrow aplasia and 1 of cerebrovascular accident. 3 patients are alive with no evidence of disease. 2 had microscopic potentially platinum sensive tumours, the third had extensive retroperitoneal and liver metastases which had developed during first-line treatment. This subject remains free of disease 84 months after second-line treatment.

Myelotoxicity represented the most common and serious side-effect of treatment, with 5 patients having grade 4 myelotoxicity and one had to stop treatment after the first course. One of these patients died of bone marrow aplasia 11 months after treatment.

The evaluation of regimens for second-line treatment in ovarian cancer is difficult. However, the classification of patients into well defined categories allows some objective comparisons. Our population was homogeneous with respect to the parameters proposed by Blackledge and associates [3] as all subjects had advanced disease at first diagnosis and none had disease-free interval.

As expected, the response rate among patients with measurable–evaluable tumour was higher (33%) for those patients with potentially platinum responsive tumours. The response rate of 10% in platinum refractory tumours is in the range observed with other regimens [5–7] but the most impressive response was observed in a woman with diffuse metastases which developed during cisplatin-based first-line chemotherapy.

This regimen does not require prolonged infusions. Nevertheless, it requires i.v. administration of etoposide for 3 consecutive days, therefore the administration on an outpatient basis is difficult for patients living far from the hospital. The toxicity is acceptable but the occurrence of a late bone marrow aplasia remains a matter of discussion.

The mechanism of action of doxorubicin, etoposide and cyclophosphamide differs from that of cisplatin and of taxol, therefore this second-line treatment retains a possible usefulness in patients refractory to regimens including the aforementioned drugs.

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