

SHORT COMMUNICATION

Oral contraceptives, reproductive factors and *p53* gene expression in colorectal cancer

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Protective effects of oral contraceptives and high parity on the development of colorectal cancer have been hypothesized. However, the epidemiological data are inconsistent. This inconsistency may be due in part to the biological heterogeneity of colorectal tumors. A recent investigation of hepatocellular carcinoma demonstrated an association between lack of *p53* expression and oral contraceptive use. We investigated the relationship between oral contraceptive use and other reproductive factors with *p53* over-expression in 64 post-menopausal women, 45-86 years of age, with non-familial colorectal adenocarcinoma. Fifty per cent (32/64) of colorectal tumors displayed nuclear over-expression of *p53* protein. Women with a history of oral contraceptive use were significantly less likely to have *p53* positive (+) tumors than women who never used oral contraceptives ($P = 0.02$). In contrast, tumors from women who had never been pregnant were more likely to be *p53*+ compared to tumors from parous women ($P = 0.10$). These data suggest that oral contraceptive use and pregnancy are associated with a *p53* independent pathway in the development of colorectal cancer.

The suggestion that reproductive factors influence the development of colorectal cancer in women originated with the observation of Fraumeni *et al.* (1) that nuns experience an excess risk of large bowel, as well as breast cancer. McMichael and Potter have described the epidemiological and biological evidence supporting a hormonal role in the development of colon cancer (2,3), and hypothesized protective effects for high parity and use of oral contraceptives (2). The mechanism to explain an effect of hormones on colorectal cancer risk remains unclear, but may involve effects on bile acid metabolism or molecular alterations brought about by changes in the hormonal milieu of the colon (4).

The study of *p53* alterations may provide greater insight into the carcinogenic pathway and the etiologic role played by various exogenous and endogenous factors (5,6). In humans, both mutations and over-expression of *p53* have been associated with environmental carcinogens (7) including, exposure to cigarette smoking in lung (8) and bladder cancer (9), UV light in squamous cell carcinoma (10) and aflatoxin B₁ in hepatocellular cancer (11).

A relationship between female hormones and *p53* gene expression in cancer is supported by the consistent association in multiple studies between *p53* protein expression and

estrogen receptor negativity in breast tumors (12). Moreover, De Benedetti *et al.* (13) recently reported a low prevalence of *p53* mutation and over-expression among 11 cases of hepatocellular cancer who used oral contraceptives. We have previously investigated epidemiological risk factors and *p53* gene expression in a case-control study of colorectal cancer (14,15). To determine whether our data support the hypothesis proposed by De Benedetti *et al.*, we analysed the association of oral contraceptives and reproductive factors with *p53* over-expression in a series of colorectal carcinomas.

The current study is part of a larger investigation comprising 163 cases (91 males, 72 females) and 326 controls (182 males, 144 females). Details of the study population have been reported previously (14,15). Eligible cases were restricted to 64 post-menopausal women 45-86 years of age (mean = 65.5; SD = 10.3) with non-hereditary primary adenocarcinoma of the colon or rectum. Cases completed epidemiological questionnaires concerning their reproductive history and use of exogenous hormones.

Materials and methods

All patients' tumors were analysed immunohistochemically for *p53* protein over-expression by the avidin-biotin immunoperoxidase method using polyclonal anti-*p53* antibody NCL-*p53*-CM1 (Novocastra Laboratories, Inc., Ltd., UK) (16). Sections known to stain positively were included in each run, receiving either primary anti-*p53* antibody or PBS, as positive and negative controls, respectively. Details of the immunohistochemical methods used for this study have been previously described (14,15).

The slides were examined and scored independently by two pathologists without any clinical or pathological information. Discrepant findings by these pathologists were infrequent and were resolved by joint review. Tumors were classified as *p53* negative (-) if 0-19% of cells displayed positive nuclear staining, and *p53*+ if at least 20% of tumor cells were positive for nuclear *p53*. Similar classification schemes have been used in other molecular epidemiological investigations of *p53* over-expression (17-19).

Results

Fifty per cent (32/64) of colorectal tumors displayed nuclear over-expression of *p53* protein. Fisher exact tests were used to calculate *P*-values. Neither age, race, education, body mass index, TNM stage, nor tumor mucin character, location or differentiation differed with respect to *p53* over-expression. Patients with a positive family history of colorectal cancer were more likely to have *p53*- tumors compared to those

Table I. Oral contraceptive use and parity by *p53* gene expression

Factor	<i>p53</i> + Cases (n = 32)	<i>p53</i> - Cases (n = 32)	<i>P</i> -value
Oral contraceptive use			
ever	2 (17%)	10 (83%)	
never	30 (58%)	22 (42%)	0.02
Parity			
nulliparous	6 (86%)	1 (14%)	
parous	26 (46%)	31 (54%)	0.10

without a family history ($P = 0.11$). Women with a history of oral contraceptive use were significantly less likely to have p53+ tumors than women who never used oral contraceptives ($P = 0.02$; Table I). The average duration of oral contraceptive use was 3.6 years and ranged from 6 months to 15 years. Cases began use of oral contraceptives between 24 and 41 years of age. The two cases with p53+ tumors had used oral contraceptives for 1 and 6 years' duration. In addition, tumors from women who had never been pregnant were more likely to be p53+ compared to tumors from parous women ($P = 0.10$) (Table I). p53 gene expression did not differ with respect to menopausal estrogen use or age at first birth (data not shown).

Discussion

Evidence indicates that oral contraceptives and pregnancy reduce secondary bile acid concentrations (20), possibly lowering carcinogenic potential. Bile-acid induced carcinogenesis is hypothesized to involve DNA damage resulting from toxic effects of bile acids on colonic mucosa (21). However, there is little epidemiological support for a protective effect of oral contraceptives with only two studies showing clear reductions in risk (22,23). Several studies have reported lower risks for multiparous compared with nulliparous women, while others have found no association with parity (24). There may be several explanations for the inconsistency in epidemiological studies including the molecular heterogeneity of colorectal tumors, in particular, p53 gene expression.

To our knowledge, this is the first report examining the association between oral contraceptives and p53 gene expression in colorectal cancer. Our results for colorectal cancer are similar to those of De Benedetti *et al.* (13) for hepatocellular tumors in observing a low prevalence of p53 over-expression among oral contraceptive users. In addition, we observed a higher prevalence of p53 gene expression among nulliparous women, none of whom had used oral contraceptives, than among parous women. Lower bile acid concentrations in oral contraceptive users and multiparous women may explain their increased probability of developing p53- tumors. A recent *in vitro* study showed that normal colonic mucosa exposed to low bile salts concentrations demonstrated increased apoptotic cell death, a pathway associated with p53- colorectal tumors (25,26).

Our findings should be considered as preliminary and interpreted with caution. This analysis was based on a small number of cases. Biases inherent in this type of study design could explain our results, particularly confounding factors such as smoking and diet. It is interesting, however, that our study is consistent with that of De Benedetti *et al.* in suggesting that p53 molecular pathways in the etiology of some cancers may be associated with specific reproductive and hormonal factors. A larger investigation of hormonal factors and somatic alterations in hormone-related cancers is warranted.

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