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Type I Insulin-like Growth Factor Receptors in Human Colorectal Cancer

A. Adenis, J.-P. Peyrat, B. Hecquet, A. Delobelle, G. Depadt, P. Quandalle, J. Bonneterre and A. Demaille

Type I insulin-like growth factor (IGF) receptors have been recently characterised in human colorectal cancers. The aim of this study was to determine whether type I IGF receptor concentration may be related to prognostic variables in colorectal cancers. Saturation experiments with [^{125}I] IGF-I were performed on membrane preparations of 46 frozen specimens (20 tumours, 26 controls) and analysed according to the Scatchard method. In all the studied cases, we found a single class of high affinity binding sites in both normal and malignant colorectal tissues (median 0.17 and 0.15 nmol/l, respectively). Using paired analysis, we found no significant difference in terms of type I IGF receptor concentration between malignant and normal colorectal tissues. There was also no relationship between type I IGF receptors and any of the tumour characteristics studied. This study does not support a critical role of the type I IGF receptors in the clinical management of colorectal cancers.
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INTRODUCTION

COLORECTAL CANCER is one of the most prevalent tumours observed in Western populations. Several recent papers focused attention on genomic alterations during colorectal tumour development (for review see [1, 2]; however, little is known on the mechanisms involved in colorectal cancer growth. IGFs (insulin-like growth factor I and II) are important factors implicated in proliferation of different cell types [3]. IGF-I, also termed somatomedin C, contributes together with growth hormone (GH), to skeletal development via the endocrine pathway [4, 5]. This factor stimulates the growth of various colorectal cancer cell lines [6–11]. IGF-II, which is moderately GH-dependent, is able to stimulate the growth of at least 5 colorectal cell lines [8].

IGFs can act via the endocrine pathway but, as they are synthesised locally in many different tissues [3], including the liver and the gastrointestinal tract [12–14], they can also act via autocrine or paracrine pathways. Their messenger RNAs have been found in the intestine [13, 15, 16].

The first step of IGFs' action is their binding to membrane type I and type II receptors. The type I IGF receptor is remarkably similar to that of insulin, comprising an heterotetrameric structure with two α and two β subunits joined by disulphide bridges [17]. On several different cultured animal and human cancer cell lines, as well as fresh tissues, the characteristics of the IGF-I binding sites assessed by competitive binding and cross-linking are those of the type I receptor (for review see [3, 18]). This receptor is able to recognise not only IGF-I, but also IGF-II and to a lesser extent insulin [7, 19]. Type I IGF receptors have been also characterised, in both rodent [20–22] and human intestinal cancer cell lines [6–8, 23–25], in both rodent [26–28] and normal colonic tissues [29], and recently in human colorectal cancers [7]. Type II IGF receptor has been described not only in animal colonic cell lines [24], but also in rodent [26, 27] and human [29] colorectal tissues. It appeared that the extracellular domain of this receptor might be secreted in the culture media [30]. A number of IGF binding proteins (IGF BP), structurally different from the IGF receptors, has also been found in the culture media of all the colorectal cells being studied [30].

The type I IGF receptor is an important step in the action of IGF-I, as well as IGF-II, on the growth of colon cancer cells, comprehensively reviewed by Singh and Rubin [30]; the level of these receptors might be a marker for determining the sensitivity of these tumours to IGFs.

In the present study, we determined the concentrations of type I IGF receptors in a sample of 20 human colorectal carcinomas; furthermore, we attempted to relate type I IGF receptor status to usual prognostic variables.

PATIENTS AND METHODS

Patients

20 patients (female 9, male 11) were included in the study; the median age was 66.5 years (range 37.8–81). The patients were operated on for primary or metastatic colorectal carcinoma and their tumours removed (colon 15, rectum 5). Detailed patient characteristics are shown Table 1. In all patients, the adjacent, normal colorectal tissue was also available for the study. In addition, we also obtained normal colorectal tissues from 6

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Correspondence to A. Adenis at the Département d'Oncologie Médicale. A. Adenis and J.-P. Peyrat are at the Laboratoire d'Oncologie Moléculaire Humaine, A. Adenis J. Bonneterre, and A. Demaille are at the Département d' Oncologie Médicale, A. Delobelle is at the Département Anatome-Pathologie, G. Depadt is at the Département de Chirurgie, B. Hecquet is at the Unité de Biostatistique, Centre Oscar Lambret, BP 307, 59030 Lille, and P. Quandalle is at the Département de Chirurgie, Centre Hospitalier Régional et Universitaire, Lille, France.
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Table 1. Tumour and patient characteristics

Patient	Sex	Age (years)	Tumour site	TNM	Differentiation	DFS (days)	OS (days)	IGF1-R status
1	M	75.4	Colon	400	Well	1185+	1185+	+
2	F	66.5	Colon	210	Well	1167+	1167+	−
3	M	70.4	Colon	320	Moderate	1125+	1125+	−
4	F	67.8	Colon	411	Well	NO	107	+
5	F	81	Colon	400	Moderate	1140+	1140+	+
6	M	63.6	Colon	410	Moderate	700	700	−
7	M	71.8	Rectum	300	Well	1093+	1093+	+
8	F	60.6	Rectum	400	Well	912+	912+	+
9	F	64	Colon	420	Well	NO	634	+
10	F	77.8	Colon	410	Well	425	527	+
11	F	66.9	Colon	301	Well	NO	114	−
12	M	53.4	Rectum	410	Well	780+	780+	−
13	M	80	Rectum	300	Well	795+	795+	−
14	M	67	Colon	300	Well	210	718+	+
15	M	71.4	Colon	211	Well	NO	425+	+
16	F	66.3	Colon	431	Moderate	NO	194	+
17	M	37.8	Rectum	421	Well	NO	270	+
18	F	58.3	Colon	200	Well	459+	459+	+
19	M	65.6	Colon	401	Well	NO	238	+
20	M	41.2	Colon	401	Well	NO	446+	+

NO, no occurrence; DFS, disease-free survival; OS, overall survival.

patients with various neoplasms (unknown origin 1, carcinoid 1, stomach 1, colorectal 3); which resulted in a control group of 26 normal colorectal specimens (female 14, male 12; median age 66 years (range 37.8–81)). No patient had pre-operative radiation therapy.

Collection of tumours

Immediately after surgical removal, resected tumour and normal colorectal tissues were opened, then washed in cold 0.9% saline. Incisional biopsies of macroscopically viable tumours were removed, and the adjacent tissues processed for routine histopathology. The tumours were classified according to the TNM classification [31]. There were 1 stage I, 6 stage II, 5 stage III and 8 stage IV. Macroscopically normal colorectal tissues from a site distant (more than 5 cm) from the tumour were also removed as control tissue. All the samples were finely divided, snap frozen in liquid nitrogen and stored at −80°C until assayed.

Tissue processing

The frozen tissue was weighed and then pulverised (Spex-Bioblock, France). The tissues were homogenised in 20 mM Tris,3 mM EDTA, 1 mM dithiothreitol, 0.01% azide, pH 7.6. The homogenate was ultracentrifuged at 105 000 *g* for 60 min. The supernatant (cytosol) was removed and the pellet (microsomal fraction) was resuspended in 25 mM Tris–HCl, 10 mM $MgCl_2$, 10^{-4} M phenylmethylsulphonyl fluoride buffer, pH 7.6. The protein concentration was determined in the microsomal fraction by the method of Lowry and associates [32].

IGF-I labelling

Human synthetic IGF-I was purchased from Amersham (ARN 4010; Amersham-France, Paris, France). A modification [19] of the method of Hunter and Greenwood, using 800 ng chloramine-T and 1 μg IGF-I, with incubation for 50 s at 23°C, was used to iodinate IGF-I. Iodinated IGF-I was purified on an ACA-54 column (LKB, France), and the tubes comprising the radioactive protein peak were diluted in assay buffer, and could be stored as long as 2 weeks at 4°C. Specific activities, as calculated by self-displacement analysis [33], ranged between 160 and 220 μCi/μg [19].

Type I IGF receptor concentration assays

For receptor determinations, membrane proteins (400 μg) were incubated, in all the cases, for 5 h at 4°C with seven increasing concentrations (0.01–1 nM) of iodinated IGF-I in the presence or absence of an IGF-I crude preparation; the optimal binding assay conditions in terms of time and temperature were defined in a previous study [34]. The final incubation volume was adjusted to 0.5 ml with Tris–$MgCl_2$ buffer containing 0.1% bovine serum albumin (fraction V, Ref. A3912; Sigma Chemical Company, St Louis, Missouri, U.S.A.). The results were analysed according to the Scatchard method [35], which gave the equilibrium dissociation constant, *Kd*, and the total number of specific binding data expressed as fmol/mg proteins. In each series, a characterised pool of cell membrane receptors from the breast cancer cell line, MCF-7, was included for quality control.

Moreover, in an attempt to compare these results with data obtained by our group in breast cancer [19] and in benign breast disease [36], we also expressed the number of type I IGF receptors as the percentage of the total radioactivity/400 μg of membrane protein. We used 1% as the positive threshold, since when the assay was performed on boiled membranes, i.e. on membranes with denatured receptors, the difference between the binding in the absence or in the presence of an excess of IGF-I was always less than 1% [19].

Finally, to be sure that our binding was type I IGF receptors and not binding proteins, we inhibited the binding of IGF-I to its receptor by addition of α-IR3 (Cliniscience, France), a mouse monoclonal antibody that is highly specific for type I IGF receptor [37].

Statistical analysis

As the distribution of type I IGF receptor concentrations could not be established as normal, non-parametric tests were

used. Results were presented as median, lower and upper values. Differences between groups were assessed using the Mann–Whitney U-test for unpaired data and the Wilcoxon's rank sum test for paired data. The Spearman rank test was used for correlation. P values of less than 0.05 were considered significant.

RESULTS

Binding sites

Binding inhibition by α-IR3 was observed in all cases (Figure 1). The Scatchard analysis of the binding data of saturation of each malignant and normal colorectal specimen with increasing concentrations of [^{125}I] IGF-I, revealed a single class of high affinity binding sites (median 0.17 and 0.15 nmol/l, respectively). One representative of these experiments is shown (Figure 2). The standard error on each type I IGF receptor concentration was low; the mean variation coefficient for all 14 point Scatchard determinations was 12%.

Type I IGF receptor level

The median number of IGF-I binding sites in normal colorectal tissues (n=26) was 8 fmol/mg membrane protein (range 0–39) and 11 fmol/mg membrane protein (range 4–57) in malignant colorectal tissues (n=20). Using paired-analysis, we found no significant difference (n=20; P >0.05) between the type I IGF receptor concentration in the malignant colorectal cancers and in the normal colorectal tissues. The distributions of type I IGF receptor concentrations, in normal colorectal tissues and in colorectal cancers, are shown Figure 3a and 3b. The specific binding was expressed as the percentage of the total radioactivity (200 000 cpm) added to 400 μg of membrane protein; this allowed the comparison with our previous studies [19]. Forty-six per cent (12/26) of normal and 70% (14/20) of malignant colorectal tissues bound more than 1% of the total labelled IGF-I, and therefore were considered positive (Figure 3c and 3d). We obtained the same percentage of positive tumours with regard to type I IGF receptor concentration, taking 7 fmol/mg protein as the positive threshold.

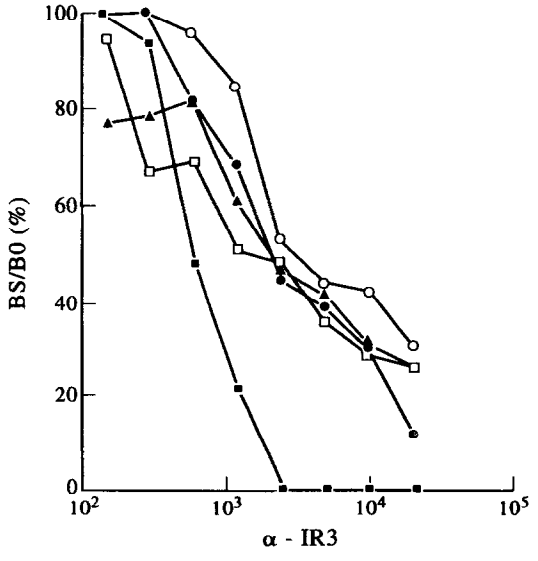

Figure 1. Binding inhibition by the mouse monoclonal antibody α-IR3 that is highly specific for type I IGF receptors. Specific binding (BS) is expressed as the percentage of that observed in the absence of the α-IR3 antibody (B_0). Five cases shown.

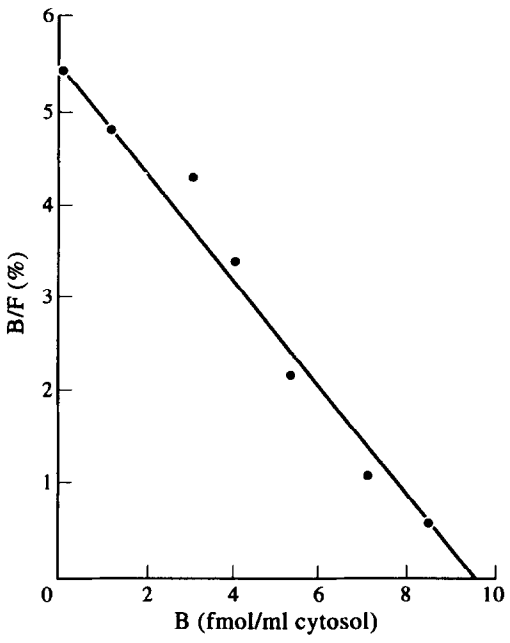

Figure 2. Example of type I IGF binding to membrane specific receptors from one colorectal tumour (Scatchard analysis, *Kd*= 165 pM/l and *n*= 9.6 fmol/mg proteins).

Relation between type I IGF receptors and clinical features

A correlation between age and the number of IGF-I binding sites was found in normal colorectal tissues (P<0.02), but not in colorectal cancers. In our population, there was no relationship between type I IGF receptors (number of site or *Kd*) and any of the usual clinical prognostic parameters of the TNM classification (data not shown). When studying the relationship between the level of type I IGF receptors and the degree of transmural invasion expressed by the T, the P value was 0.08.

DISCUSSION

In this study, we showed that α-IR3 antibodies compete with IGF-I binding (Figure 1), demonstrating that these sites are type I IGF receptors, confirming Guo and asssociates' results that demonstrated that IGF-I binding was on type I IGF receptors [7]. Our results also confirmed that type I IGF receptors are found in normal and malignant human colorectal tissues [7, 29]. Moreover, our results suggest that human colorectal tissues are not characterised by higher type I IGF receptor concentration than normal human colonic samples. In fact, *in vitro* studies have previously shown the presence of high affinity binding sites for IGF-I both in rodent [20–22] and in human colon cancer cells [6–8, 23–25]. *In vivo*, characterisation of IGF-I binding sites demonstrated type I IGF receptors not only in normal rodent colon [26–28], but also in normal human colonic tissues [29] and in three freshly resected human colon cancers [7].

The *Kd* values we observed for our 20 primary human colon cancers (0.17 nmol/l) were similar to values reported by Guo and colleagues in three human colon cancers (0.12 nmol/l) [7]. In addition, these figures did not differ from the *Kd* values we obtained in normal human colonic tissue (0.15 nmol/l) although they were far lower than those obtained by Rouyer-Fessard and associates (8.6 nmol/l) [29]. The concentration of type I IGF receptors that we found on colonic tissues—malignant or normal—(8–11 fmol/mg membrane protein) is much lower than that reported by Rouyer-Fessard and colleagues on normal

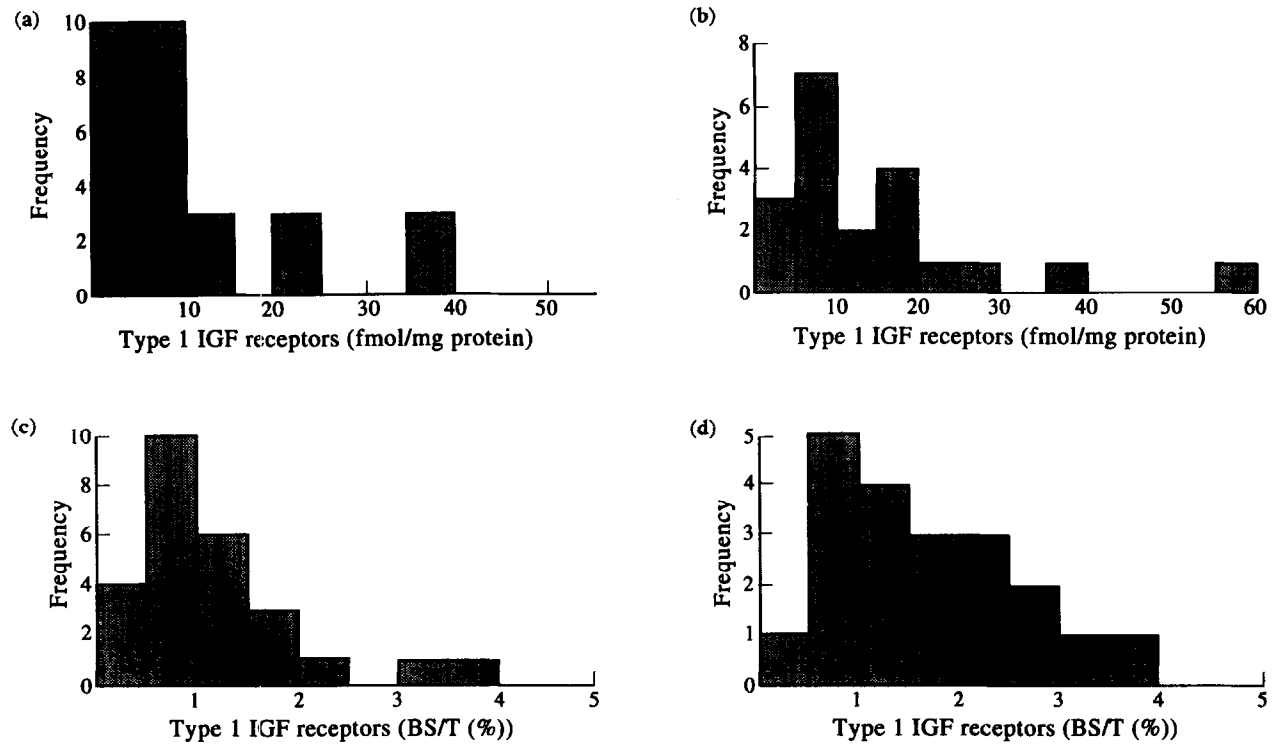

Figure 3. Distribution of type I IGF receptor concentrations (expressed as fmol/mg proteins) in (a) normal and (b) tumour colorectal tissues. Distribution of type I IGF receptor concentrations, when expressed as the ratio of the specific binding (BS) of labelled IGF-I to its receptor on the total amount of added radioactivity (T), in (c) normal colorectal tissues and in (d) colorectal cancers.

human colon membranes (800 fmol/mg membrane protein) [29], and by Guo and associates in human colonic cancer specimens (250 fmol/mg membrane protein) [7]. Several hypotheses could account for the discrepancy between our results and the Guo and Rouyer-Fessard papers [7, 29]. Firstly, it is worthwhile to note that in the papers by Guo and Rouyer-Fessard [7, 29] the method of calculating iodinated IGF-I specific activity was not indicated. If iodinated IGF-I specific activity is calculated by isotope recovery, then the specific activity might be underestimated because of the presence of inactive IGF-I, and subsequently the number of binding sites might be overestimated. Secondly, in Guo's paper [7], the *Kd* and the number of binding sites were derived from a competitive curve, while in our present study saturation experiments were done in each of the cases. Deriving *Kd*s and the number of sites from competitive curves lead, in our experience [38], to an overestimation of these parameters, which can range from two to 10 times more. Thirdly, the similarity in *Kd*s that we found in the normal and malignant human colorectal tissues does not support the hypothesis that malignant transformation could be associated with an increasing affinity to type I IGF receptors, as suggested by Guo and colleagues [7]. Finally, as type I IGF receptors are negatively regulated by IGF-I levels [39], we cannot exclude the possibility that low type I IGF receptor concentrations may result from elevated autocrine and/or endocrine IGF-I which may be linked, for example, with differences in nutritional status and/or age [40, 41]. Interestingly, we found, in normal colorectal tissues, a correlation between type I IGF receptor levels and the age of patients. A high type I IGF receptor level was associated with aging, this was not found in malignant colorectal samples. We, therefore, can speculate that type I IGF receptors, expressed by malignant tissues, could be less sensitive than normal colorectal tissues with regard to hypothetical down-regulation processes induced by plasma IGF-I levels. In fact, it has been shown that age affects the plasma concentration of IGF-I [41]. In addition, our group recently established that breast cancer is associated with higher plasma IGF-I levels as compared with controls of the same age range [41].

The precise localisation of the type I IGF receptors in human colorectal tumours and in normal colonic tissues is not known. Pillion and colleagues have shown that type I IGF receptors are present on apical membranes from rabbit colon epithelial cultured cells [23]. Additionally, autoradiographic studies of IGF-I binding demonstrated, in the rabbit colon, the presence of type I IGF receptors in both mucosal and muscular layers [28]. In our experience, the use of the α-IR3 antibody in immunohistochemical experiments indicated preferential staining in the epithelial enterocyte layer and to the malignant epithelial cell compartment (data not shown).

We found no statistical difference in concentrations of type I IGF receptor in malignant and normal human colorectal tissues. Differences between type I IGF receptor concentrations in normal tissue, benign tumour and cancers have been previously described in breast tissues [19, 42, 43]. In contrast to our experience in breast cancer [44], we did not find any correlation between type I IGF receptors and the usual prognostic characteristics. Of course, we cannot exclude an independent prognosis value of type I IGF receptor concentrations that may appear with longer follow-up. It is worth noting that, in a small series reported by Zenilman and associates, no relationship between type I IGF receptor expression and tumour stage, assessed by the Dukes classification, was found [45]. Finally, the lack of difference between the type I IGF receptor concentrations in the malignant human colorectal tissues and in the normal colorectal tissues may explain this absence between these receptors and the prognostic variables.

In conclusion, we found that 70% of colorectal tumours were positive for type I IGF receptors; consequently, the growth of these cancers can be stimulated by IGF-I as well as IGF-II. As we found no statistical difference in terms of type I IGF receptor concentrations between malignant and normal human colorectal tissues, it might be difficult to justify that an IGF-lowering drug may be of therapeutical interest in the treatment of colorectal cancer, as it has been suggested. This may partly explain the lack of efficiency, in human metastatic colorectal cancers, of somatostatin analogues which may induce IGF-I decrease [46].

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A Recent Increase in the Incidence of Prostatic Carcinoma in a French Population: Role of Ultrasonography and Prostatic Specific Antigen

F. Ménégoz, M.Colonna, C. Exbrayat, M. Mousseau, H. Orfeuvre and R. Schaerer

Between 1979 and 1990, the incidence rate (World Standard) for cancer of the prostate in the region of Isère (France) increased from 22.1 to 45.0 cases per 100 000 men, although there was no concurrent increase in mortality (16.0 to 17.6 cases per 100 000 men). This represents a mean increase per year of 6.3% for incidence, compared with 1.3% (NS) for mortality. Incidence of cases with metastases at diagnosis also remained stable with time. In this area, Prostatic Specific Antigen assays began in 1987, and rectal ultrasonography was implemented in 1984, but activity peaked only in 1988. Thus, during 1986–1988, there was both an implementation of new diagnostic procedures and an increase in the incidence of prostatic carcinoma, which suggests that the latter was the result of increased detection of small latent carcinomas. This has implications for public health since apart from increasing costs, it might unduly disturb the life of otherwise healthy people.

Key words: prostatic cancer, epidemiology, incidence, mortality, cancer registry, diagnostic procedures, localised cancer, metastatic cancer, cost, public health

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INTRODUCTION

INCIDENCE AND mortality rates from prostate cancer are highly variable among different countries and ethnic groups [1–3]. More recently, a marked increase in incidence has been described, raising the question of whether the risk, diagnostic measures (or reporting attitudes), or both, have changed [4–6]. Lu-Yao and Greenberg [7] believe that, in the U.S.A., the increased incidence might be a consequence of new diagnostic procedures rather than a true increase in occurrence. This paper explores this issue in a French population.

Prostate cancer risk has been shown to be correlated with some food, sexual behaviour and a high intake of meat and a low consumption of vegetables and vitamin A [3]. Precocity and frequency of sexual intercourse have been found in other studies, and this might explain a lower incidence among Roman Catholic priests [8]. A genetic predisposition is strongly suggested by a higher frequency in some families and in some ethnic groups, e.g. negroid men in the U.S.A. Non-random chromosomal deletions in prostate cancer cells, some of them in the same chromosomes as well known anti-oncogenes, have been described [5]. However, none of these factors would yield a satisfactory explanation for such an observed increase in incidence rates as those that have been described [2, 4–7].

MATERIALS AND METHODS

Isère Cancer Registry is a population-based registry [1], covering a mixed urban and rural area with a population of 1 014 000 people (1990 census). The mean incidence per year for all cancers between 1983 and 1987 was of 1500 new cases for men and 1200 for females, with incidences, respectively, of 280

Correspondence to F. Ménégoz.
F Ménégoz, M. Colonna, C. Exbrayat and R. Schaerer are at the Registre du cancer de l'Isère, 21 chemin des Sources, 38240 Meylan; M. Mousseau, H. Orfeuvre and R. Schaerer are at the Service d'Oncologie Médicale, Centre Hospitalier Regional de Grenoble, France.
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