cases in the elderly in the regions from which the overall estimates have been extrapolated. As data from France are for a 5-year former calendar period, this comparison may be somewhat approximate.

The comparison of the incidence estimates presented in this report with recently published data [5] allows us to rank Switzerland in relation to other European countries, especially those of the European Community, which are represented by no fewer than 43 national or regional cancer registries. Approximately half of these regions are of southern Europe and France; the others are situated in central and northern Europe. Such geographical distribution thus covers a large spectrum of cultural behaviours which may influence cancer rates. As far as overall incidence rates are concerned, Switzerland is in the upper half of the distribution, ranking 13th for males and 15th for females (although rankings tend to vary according to individual cancer sites). For cancers linked to alcohol consumption, such as those of the oral cavity, Switzerland occupies an intermediate rank (21st) for males and a lower one for females (34th). The incidence of stomach cancer is in the lower third of the distribution for both sexes (31st and 32nd, respectively), and that for colorectal cancers is somewhat higher (22nd and 28th, respectively). Cancer of the pancreas is relatively frequent (15th and 9th highest, respectively). Over the period covered by this study, Switzerland had a relatively favourable lung cancer ranking (28th and 25th, respectively). In contrast, the country is in an unfavourable position as regards cancers of the breast (9th), prostate (first), and cutaneous melanomas (first rank in both sexes). For the two latter cancers, however, the Swiss rank may be explained in terms of a high detection rate.

1. Parkin DM, Stjernwärd J, Muir CS. Cancer control. Estimates of the worldwide frequency of twelve major cancers. *Bull WHO* 1984, **62**, 163–182.
2. Parkin DM, Läärä E, Muir CS. Estimates of the worldwide frequency of sixteen major cancers in 1980. *Int J Cancer* 1988, **41**, 184–197.
3. Benhamou E, Laplanche A, Wartelle M, *et al. Incidence des Cancers en France*, 1978–82. Paris, INSERM, 1990.
4. Jensen OM, Estève J, Møller H, Renard H. Cancer in the European Community and its Member States. *Eur J Cancer* 1990, **26**, 1167–1256.
5. Parkin DM, Muir CS, Whelan SL, Gao YT, Ferlay J, Powell J, eds. *Cancer Incidence in Five Continents*, Vol. VI. IARC Scientific Publication No. 120. Lyon, International Agency for Research on Cancer, 1992.
6. World Health Organisation. *International Classification of Diseases*, 8th Revision. Geneva, World Health Organisation, 1967.
7. Verdecchia A, Capocaccia R, Egidi V, Golini A. A method for the estimation of chronic disease morbidity and trends from mortality data. *Stat Med* 1989, **8**, 201–216.
8. Office Fédéral de la Statistique (OFS) et Association Suisse des Registres des Tumeurs (ASRT). *Mortalité Cancéreuse. Qualité des Données en Suisse*. Contributions à la statistique suisse/15e fascicule. Berne, Office fédéral de la statistique, 1984.

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MDM2 Gene Amplification in Human Breast Cancer

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The MDM2 gene is a gene whose product binds to p53 and regulates its functions. The amplification of the MDM2 gene has been found in one third of human sarcomas, and a differential expression of MDM2 gene in relation with oestrogen receptor status was recently found in human breast cancer cell lines. We analysed 60 breast cancers for MDM2 gene amplification by Southern blot. This event was observed in 1 case with high levels of oestrogen receptor (ER). Thus, MDM2 gene amplification seems to be a rare event in breast cancer. Further studies are needed to define precisely the relationship between MDM2 amplification and ER status.

Key words: MDM2, gene amplification, breast cancer, oestradiol receptor
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THE p53 gene is a tumour suppressor gene whose inactivation through point mutations and/or deletion is frequent in solid tumours [1]. In primary breast cancer, about 20% of the cases have a p53 gene mutation [2, 3].

Although inactivation of p53 usually results from point mutations, other mechanisms of inactivation of this protein may occur in human tumours. One of those recently identified results from over-expression or amplification of the MDM2 gene. The gene was originally isolated from a tumorigenic mouse fibroblast cell line containing double minutes, a cytogenetic hallmark of gene amplification [4]. Over-expression of the MDM2 gene in cells increases their tumorigenic potential. Binding of the 90-kD MDM2 protein to the p53 protein inhibits the p53-mediated transactivation of a gene with a p53-responsive element [5]. Moreover, the p53 protein can transactivate MDM2 gene expression in an autoregulatory feedback loop [6]. The fact that

the MDM2 protein sequence contains two zinc fingers suggests that a transactivation activity could exist [5].

The human homologue MDM2 gene has been mapped to chromosome 12q13-14, and was found amplified in one third of human sarcomas, including malignant fibrous histiocytoma, rhabdomyosarcoma, osteosarcoma and liposarcoma [7–9]. Occurrence of MDM2 amplification in other human tumours has still not been extensively investigated [10]. One recent study reported a differential expression of the MDM2 gene in human breast cancer cell lines, and found a strong correlation between oestrogen receptor (ER) status and the MDM2 RNA levels; no amplification of the MDM2 gene was seen in these cell lines [11].

Gene amplification is a frequent finding in human breast cancer, usually involving the c-*myc* gene or c-*erb*B-2 and *int*-2 genes [12]. In order to determine if MDM2 gene amplification can occur in human breast cancer, we looked for this mechanism in 60 patients.

PATIENTS AND METHODS

Patients

60 cases with breast cancer were analysed after informed consent; 24 of them were node-negative patients. DNA was extracted from specimens from patients who underwent surgery at the Centre Oscar Lambret.

Methods

Southern blot analysis was performed by conventional methods after DNA digestion with *Eco* RI restriction enzyme [13]. The MDM2 probe used was a 1.7-kb cDNA probe, kindly provided by Vogelstein (John Hopkins Oncology Center, Baltimore, U.S.A.). The SA1 osteosarcoma cell line kindly provided by Shapiro (St Jude Children Cancer Research Center, Memphis, U.S.A.) was used as positive control. Blots were rehybridised with D12S2 (American Type Culture Collection 57181), an anonymous probe located on chromosome 12 [8]. The band intensities was evaluated by scanning (Cliniscan, Helena Laboratories, France).

In all 60 patients, oestradiol receptor (ER) and progesterone receptor (PgR) assays were determined by the dextran-coated charcoal method, as described previously [14]. Our laboratory is affiliated with the European Organization for Research and Treatment of Cancer Receptor Study Group which organises quality controls of the assays [15].

RESULTS

MDM2 gene amplification was found by Southern blot analysis in one of 60 human breast cancers analysed (Figure 1). Amplification in this patient was 7-fold relative to a normal control. No rearrangement of the MDM2 gene was seen in any patient. The patient with MDM2 gene amplification had 10 invaded nodes, a large tumour size (diameter 6.5 cm) and histologically poorly differentiated ductular carcinoma. Tumour size and node involvement of this patient were the largest of the 60 cases. ER concentration was 368 fmol/mg protein and PgR concentration was 7 fmol/mg protein.

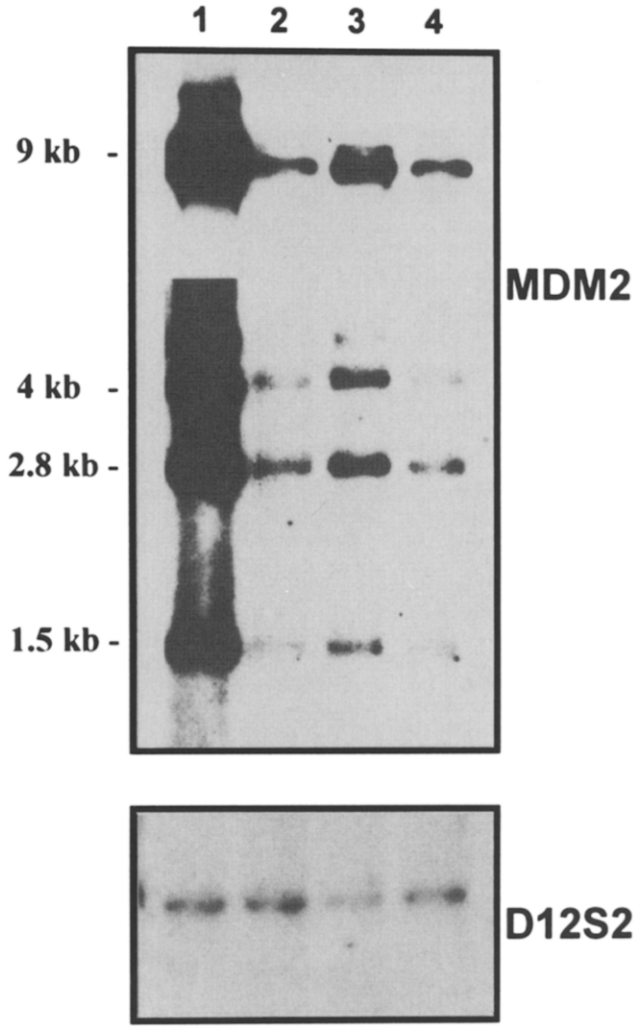

Figure 1. Southern blot analysis (*Eco*RI digestion) of the MDM2 gene in primary breast cancer. Lane 1: MDM2-amplified SA1 osteosarcoma cell line. Lanes 2 and 4: unamplified cases. Lane 3: the 7-fold MDM2 amplified case. For all the cases the signals after chromosome 12 D12S2 probe hybridisation were of similar intensity.

DISCUSSION

We found that MDM2 amplification can occur in human breast cancer. However, the frequency of this event seems to be rare. It is interesting to note that the sole amplification was observed in a rare case where node involvement and tumour size were high, and ER were positive. The relationship with ER is in agreement with the results of Sheikh *et al.* [11] who demonstrated preferential MDM2 expression in ER-positive cell lines. They also showed that when ER-negative cell lines were transfected with expression plasmid encoding human ER, an increase of MDM2 mRNA levels was observed. However, the mechanism by which ER modulates the MDM2 mRNA level remains unclear.

MDM2 gene amplification seems to be a rare event in human breast cancer. However, the real implication of the MDM2 gene in carcinogenesis must be investigated extensively. Recently, Khatib *et al.* [16] demonstrated that, in human sarcoma, the amplicon on chromosome 12 band q13 contained not only the MDM2 gene but also the GLI gene, which encodes a potential transcriptional regulatory protein with zinc finger DNA and the CDK4 gene. The CDK4 gene encodes a 34-kD cyclin-dependent kinase, a major catalytic subunit of mammalian D-type cyclins.

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The relative responsibility of each gene involved in this amplicon in carcinogenesis thus remains unknown. It is of interest to note that, in a rhabdomyosarcoma cell line, the amplicon did not include the MDM2 gene [16]. Further studies are needed to determine if one or several of these genes are also co-amplified in human breast cancer.

1. Hollstein M, Sidransky D, Vogelstein B, Harris CC. p53 mutations in human cancers. *Science* 1991, **253**, 49–53.
2. Andersen TI, Holm R, Nesland JM, Heimdal KR, Ottestad L, Borresen AL. Prognostic significance of TP53 alterations in breast carcinoma. *Br J Cancer* 1993, **68**, 540–548.
3. Thorlacius S, Borresen Al, Eyfjord JE. Somatic p53 mutations in human breast carcinomas in an Icelandic population: a prognostic factor. *Cancer Res* 1993, **53**, 1637–1641.
4. Fakharzadeh SS, Trusko SP, George DL. Tumorigenic potential associated with enhanced expression of a gene that is amplified in a mouse tumor cell line. *EMBO J* 1991, **10**, 1565–1569.
5. Momand J, Zambetti GP, Olson DC, George D, Levine AJ. The MDM2 oncogene product forms a complex with the p53 protein and inhibits p53-mediated transactivation. *Cell* 1992, **69**, 1237–1245.
6. Barak Y, Juvent T, Haffner R, Oren M. MDM2 expression is induced by wild type p53 activity. *EMBO J* 1993, **12**, 461–468.
7. Oliner JD, Kinzler KW, Meltzer S, George DL, Vogelstein B. Amplification of a gene encoding a p53-associated protein in human sarcomas. *Nature* 1992, **358**, 80–83.
8. Ladanyi M, Cha C, Lewis R, Jhanwar SC, Huvos AG, Healey JH. MDM2 gene amplification in metastatic osteosarcoma. *Cancer Res* 1993, **53**, 16–18.
9. Meltzer PS, Jankowski SA, Dalcin P, Sandberg AA, Paz IB, Coccia M. Identification and cloning of a novel amplified DNA sequence in human malignant fibrous histiocytoma derived from a region of chromosome 12 frequently rearranged in soft tissue tumors. *Cell Growth Differentiation* 1991, **2**, 495–501.
10. Preudhomme C, Quesnel B, Vachée A, *et al.* Absence of amplification of MDM2 gene, a regulator of p53 function, in myelodysplastic syndromes. *Leukemia* 1993, **7**, 1291–1293.
11. Sheikh MS, Shao ZM, Hussain A, Fontana JF. The p53-binding protein MDM2 is differentially expressed in human breast carcinoma. *Cancer Res* 1993, **53**, 3226–3228.
12. Callahan R, Campbell G. Mutations in human breast cancer: an overview. *J Natl Cancer Inst* 1989, **81**, 1780–1786.
13. Maniatis T, Fritsch E, Sambrook J. *Molecular Cloning: a Laboratory Manual.* New York, Cold Spring Harbor, 1990.
14. Bonneterre J, Peyrat JP, Beuscart R, Demaille A. Prognostic significance of insulin-like growth factor 1 receptors in human breast cancer. *Cancer Res* 1990, **50**, 6931–6935.
15. Koenders T, Thorpe SM on behalf of the EORTC Receptor Study Group. Standardization of steroid receptor assays in human breast cancer. IV: Long-term within and between laboratory variation of estrogen and progesterone receptor assays. *Eur J Cancer Clin Oncol* 1986, **22**, 945–953.
16. Khatib Z, Matsushime H, Valentine M., Shapiro DN, Sherr CJ, Look T. Coamplification of the CDK4 gene with MDM2 and GLI in human sarcomas. *Cancer Res* 1993, **53**, 5535–5541.

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Evidence Against Involvement of APC Mutation in Papillary Thyroid Carcinoma

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Papillary thyroid carcinoma (PTC) is one of several tumours associated with familial adenomatous polyposis (FAP), an inherited tumour syndrome which appears to result from germ-line mutation of the APC tumour suppressor gene. Here we investigate the possibility that somatic mutation of APC might play a role in sporadic PTC. 16 cases of PTC together with matched normal tissue were examined by single-strand conformation polymorphism (SSCP) analysis, concentrating on the mutation cluster region (MCR) of the APC gene (codons 1286–1513). No evidence of mutation was observed in any sample. We conclude that APC mutation, at least in the MCR, is not a significant causal mechanism in sporadic PTC.
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INTRODUCTION

PAPILLARY THYROID carcinoma (PTC) is the most common form of malignancy in the human thyroid. It is a differentiated neoplasm of follicular epithelium, with a distinctive morphology that occurs most frequently in women in the fourth and fifth decades of life. The aetiology of PTC is unknown, though the tumour is more common in populations with high dietary iodide intake [1] and in those who have been exposed to ionising radiation [2].

Activation of four oncogenes has so far been firmly implicated in the genesis of sporadic papillary carcinoma [3]; *ras* or *gsp*, activated by point mutation, and *ret* or *trk*, activated by chromosomal rearrangement. It has recently been shown [4], however, that these are nearly always found in isolation and are, therefore, presumably alternative rather than co-operating events. Since it is highly unlikely that any single activated oncogene can generate a clinical cancer, unknown co-operating events must be involved, which by analogy with other tumour types are likely to include loss of tumour suppressor genes. Unfortunately, cytogenetics and allelotyping have been disappointingly non-informative for PTC, the only reproducible abnormality being an inversion of chromosome 10 [5], which is now known to involve the *ret*