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GENETIC CHANGES IN SOMATOSTATIN RECEPTOR POSITIVE BREAST TUMORS

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Summary—Forty-nine primary breast tumors were analyzed for the expression of the somatostatin receptor (SSR) and genetic changes in the RB tumor suppressor gene. Twenty-four tumor samples were shown to contain receptors for somatostatin and in eight of these SSR-positive tumors we observed a mutation in the RB gene. However, since also in the group of SSR-negative tumors in eight of the 25 cases an alteration of the RB gene was observed, loss of this tumor suppressor gene is not specific for the SSR-positive subgroup of breast tumors. A similar, equal distribution between SSR-positive and SSR-negative breast tumors was observed for the six tumor samples which showed amplification of the *neu* proto-oncogene.

INTRODUCTION

The inactivation of a series of tumor suppressor genes and the activation of one or more oncogenes appear to be cumulative steps in the development of many cancers. This model is probably also valid for breast cancer and some of the genetic changes implicated in the development of breast cancer have been identified: loss of heterozygosity of the chromosome regions 1q, 3p, 11p, 13q14 and 17p13 and amplifications of the *int*-2, *myc* and *neu* proto-oncogenes [1–5].

However breast cancer is a large and heterogeneous group of tumors and as yet it is not clear whether these genetic alterations are equally important in all types of breast cancer or if, like in e.g. lung cancer, a particular combination of events is present in a histologically and clinically distinct subgroup of tumors. The finding of *neu* amplifications in predominantly the comedo type of ductal carcinoma *in situ* [5], and loss of heterozygosity of regions of chromosome 22q in lobular carcinoma [6] strongly support this latter possibility.

Approximately 20% of the breast tumors contain receptors for the peptide hormone somatostatin (SS) [7, 8]. The presence of somatostatin receptors is characteristic for neural and endocrine tumors [9, 10] and also in these breast tumors positively for SSR correlates with the presence of other markers of neuroendocrine differentiation [8]. Also clinically is this distinction relevant since patients with SSR-positive breast tumors have, compared to patients with SSR-negative tumors, a relatively good prognosis [11].

Since these tumors characterized by the presence of SSR have a differentiation pattern similar to that of typical neuroendocrine tumors, as for example pulmonary carcinoids and small cell lung carcinomas, they may also share a common mechanism in oncogenesis. These latter tumors often show loss of heterozygosity of chromosome region 13q14 (retinoblastoma gene locus) and/or amplification of members of the *myc* family of oncogenes [12, 13]. Similar genetic changes were also reported for breast tumor samples and in the presented study we investigated whether genetic changes such as mutations in the retinoblastoma (RB) gene and/or oncogene amplifications occur specifically in the SSR-positive subgroup of breast tumors.

EXPERIMENTAL

Patient material

Forty-nine primary breast tumors were obtained from the surgery departments of the Dijkzigt Academic Hospital and the Dr Daniel den Hoed Cancer Center in Rotterdam between 1984 and 1990. Immediately after removal of the tumor, one part was taken for histological examination and the remaining tumor material was snap frozen and stored in liquid nitrogen

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until use. The tumors were histologically classified according to the criteria of the World Health Organization.

Somatostatin receptor autoradiography

Somatostatin receptors were measured by autoradiography on 10 μm cryostat sections of the tumor. As a ligand an iodinated stable analogue of somatostatin ^{125}I-204-090 (Tyr^3-Sandostatin, Sandoz) was used. Incubation and washing conditions were as described [7]. Non-specific binding was determined by adding unlabeled 204-090. The expression of the somatostatin receptor was scored on a semi-quantitative scale.

Southern hybridizations

High molecular weight DNA was isolated from frozen tumor samples and from normal peripheral blood lymphocytes as described [14]. Fifteen micrograms of these DNA samples were digested with *Hind*III restriction endonuclease (Promega). The resulting DNA fragments were separated by electrophoresis on an 0.8% agarose gel (BRL), and transferred to a nylon membrane (Hybond N$^+$, Amersham) according to the supplier's recommendations. The membranes were hybridized to ^{32}P-oligolabeled probes [15], washed until 0.3 × SSC (0.45 M sodium chloride, 0.045 M sodium citrate)/0.1% $NaDodSO_4$ (or, if necessary, until 0.1 × SSC/0.5% $NaDodSO_4$) and exposed to Kodak XAR films (Kodak, Eastman) for 1–7 days. After autoradiography the membranes were stripped of the probes using 0.5% $NaDodSO_4$ (100°C, 15 min) and rehybridized.

Probes

The following probes were used: RB, a 0.9 and a 3.8 kb *Eco*R1 cDNA fragment [16]; *int*-2, a 1 kb *Bam*H1–*Kpn* 1 BK4 fragment [17]; c-*myc*, a 1.6 kb *Cla* 1–*Eco*R1 fragment [18]; L-*myc*, a 1.8 kb *Sma* 1–*Eco*R1 fragment [19]; N-*myc*, a 1 kb *Eco*R1–*Bam*H1 fragment [20]; *neu*, a 1.6 kb cDNA fragment [21] and myoglobin, a 0.6 kb *Bgl*II–*Eco*R1 pcr-made fragment [22].

RESULTS AND DISCUSSION

In order to assess a possible correlation between SSR-positivity and mutations in the RB tumor suppressor gene, 49 primary breast tumors were assayed for expression of the SSR and alterations in the RB gene. Twenty-four of the tumors were SSR-positive. This high percentage is partly due to the preselection of 10 SSR-positive tumors. However, also of the remaining 30 tumor samples which were collected consecutively, 14 (47%) showed expression of the somatostatin receptor. In four samples the SSR expression showed a heterogeneous pattern. A similar observation has been made by Reubi *et al.* [23] and a possible explanation for this high incidence of SSR-positivity could be the use of larger tumor sections which allows detection of focal expression of the receptor.

To detect alterations in the RB tumor suppressor gene two cDNA probes spanning the RB gene coding region were hybridized against *Hind*III digested DNA of the tumors. As a control *Hind*III digested DNA of normal human lymphocytes was used. In 16 (30%) tumors we detected a genetic change in the RB locus. Most of these tumors were ductal carcinomas. The alterations included rearrangements as well as deletions, and an example of such a Southern blot is shown in Fig. 1. In this blot lanes 1–8 contain *Hind*III digested tumor DNAs and lane c *Hind*III digested DNA from normal peripheral lymphocytes. Lane 5 shows a

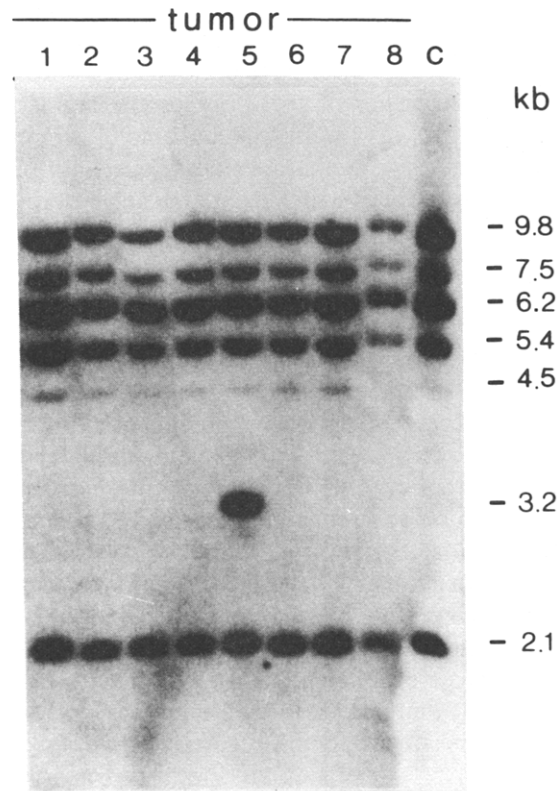

Fig. 1. RB alterations in primary breast tumors. Autoradiogram of a Southern blot containing *Hind*III digested DNA from eight primary breast tumors and control DNA after hybridization to a 3.8 kb *Eco*R1 RB-cDNA probe.

clear rearrangement of the hybridization pattern and in lane 3 the top two hybridizing fragments of 9.8 and 7.8 kb are less intense than the other hybridizing fragments. Eight of these changes were observed in SSR-positive breast tumors and the same number of RB alterations were detected in SSR-negative tumors. Although with this method it is not possible to detect all possible genetic changes in the RB gene, these results clearly indicate that presence of the SSR does not correlate with the loss of this tumor suppressor gene.

Forty-one tumors were also tested for amplification of *int*-2, the *myc* family and *neu* proto-oncogenes. In six tumors, three SSR-positive and three SSR-negative, we detected amplification of the *neu* proto-oncogene. In one SSR-positive tumor we observed an amplification of the *int*-2 proto-oncogene and in one SSR-negative an amplification of the c-*myc* proto-oncogene. Compared to the hybridization signal of the myoglobin gene, the amplifications were five to fifteen fold. No amplification or rearrangement of the L-*myc and* N-*myc* proto-oncogenes was observed. As in the case of the RB alterations, none of the observed proto-oncogene amplifications seem to be specific for the subgroup of SSR-positive breast tumors. This could mean that the SSR expressing subgroup of breast tumors has no common mechanism of oncogenesis, or that the genes involved in the etiology of these tumors are as yet not known. To answer these questions the number of investigated chromosomal regions, as well as the number of histological and clinical tumor parameters, will be extended.

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