Y₁-Mediated Effect of Neuropeptide Y in Cancer: Breast Carcinomas as Targets

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ABSTRACT

Overexpression of selected peptide receptors in human tumors has been shown to represent clinically relevant targets for cancer diagnosis and therapy. Neuropeptide Y (NPY) is a peptide neurotransmitter mediating feeding behavior and vasoconstriction. It has never been shown to be involved in human cancer. We show here, using in vitro receptor autoradiography, a NPY receptor incidence of 85% in primary human breast carcinomas (n = 95) and of 100% in lymph node metastases of receptor-positive primaries (n = 27), predominantly as Y₁ subtype, whereas non-neoplastic human breast expressed Y₂ preferentially. Y₁ mRNA was detected in Y₁-expressing tumors by in situ hybridization, whereas Y₂ mRNA was found in normal breast tissue. The strong predominance of Y₁ in breast carcinomas compared with Y₂ in normal breast suggests that neoplastic transformation can switch the NPY receptor expression from Y₂ to Y₁ subtype. Moreover, in Y₁-expressing human SK-N-MC tumor cells, an NPY-induced, dose-dependent inhibition of tumor cell growth of >40% was observed, suggesting a functional role of NPY in cancer, mediated by Y₁. NPY should therefore be added to the list of small regulatory peptides related to cancer. The high incidence of Y₁, in in situ, invasive, and metastatic breast cancers allows for the possibility to target them for diagnosis and therapy with NPY analogues.

INTRODUCTION

Regulatory peptides can be of clinical relevance, diagnostically and therapeutically, in tumors that express their respective receptors in high amounts (1–3). Indeed, it has been shown recently that selective tumors and their metastases can be precisely localized in patients by means of in vivo peptide receptor scintigraphy. This strategy was first developed for the diagnosis of somatostatin receptor-positive tumors using radiolabeled somatostatin analogues (4) with subsequent use of 125I-labeled VIP to localize VIP receptor-expressing tumors (5–7) and radiolabeled cholecystokinin/gastrin analogues to detect cholecystokinin-B-expressing medullary thyroid carcinomas (8, 9). Another clinical application has been the radiotherapeutic targeting of receptor-expressing tumors by using high doses of these radiolabeled peptides (10, 11).

The regulatory peptides with established or prospective clinical implications based on their overexpressed receptors in cancers, such as somatostatin, VIP, gastrin-releasing peptide, cholecystokinin, gastrin, and neurotensin, belong to a group of brain-gut peptides with a predominant neurotransmitter function as well as gastrointestinal and endocrine actions (12, 13). In addition to their physiological action, these peptides have been shown to play a specific role in cancer, inasmuch as they have marked effects on tumor cell growth in animal models (14). The high amount of receptors in tumors may be indicative of their pathophysiological relevance in tumor growth regulation.

NPY is a member of a family of 36 amino acid long peptides, including NPY, PYY, and PP. Human NPY has the following amino acid sequence: H-Tyr-Pro-Ser-Lys-Pro-Asp-Asn-Pro-Gly-Glu-Asp. Its main function is that of an endocrine or gut hormone, but that of a neurotransmitter; its best-known actions are at the level of the central nervous system and include stimulation of feeding behavior and inhibition of anxiety (15–17). Actions mediated by the peripheral nervous system include vasoconstriction, effects on gastrointestinal motility and secretion, insulin release, and renal secretion (18–21). The effect of NPY can be mediated by several NPY receptor subtypes, named Y₁, Y₂, of which Y₁, Y₂, and Y₅ have been extensively characterized (22). Several NPY analogues, in particular Y₁ and Y₂, antagonists, are being developed for potential clinical use to treat feeding disturbances and anxiety (23–25).

Compared with other regulatory peptides, NPY has never been associated with human cancer. In the present study, we have investigated whether there is a molecular basis for a putative NPY role in tumors and/or for the development of NPY drugs for tumor targeting. We have determined NPY receptors in one of the most frequent and harmful cancers, breast carcinoma. In more than 100 human breast tumor and metastasis samples, we have evaluated the expression at the mRNA and protein levels of the two best-investigated NPY receptor subtypes, Y₁ and Y₂, using in vitro receptor autoradiography and in situ hybridization. Moreover, we have evaluated the effect of NPY on the growth of NPY receptor-expressing SK-N-MC tumor cells in culture.

MATERIALS AND METHODS

Patient Tissues. Breast tissue samples with primary breast neoplasias were obtained from 95 patients, 36 to 91 years of age, who were operated on in several institutions. Tissue samples were kept frozen at −80°C. The diagnosis was reviewed and formulated by use of cryostat sections according to the WHO guidelines stated by Tavassoli (26). In the main group of 89 patients, 64 (72%) showed an invasive ductal carcinoma. Histological evaluation identified 49 cases with intermediate (G2), 12 cases with low (G1), and 3 cases with high (G3) grade, according to a modified Bloom-Richardson grading method (26). There were 12 invasive lobular carcinomas and 5 ductal carcinomas in situ, as well as 3 mucinous, 2 medullary, 2 apocrine, and 1 tubular carcinomas. In an additional group of six patients (four ductal and two lobular breast carcinomas), we could investigate tissue samples obtained from the primary tumor and from all of the axillary metastases. Moreover, we investigated the non-neoplastic breast tissue adjacent to the carcinoma tissue in 44 tissue samples and the breast tissue sample of one patient who had been operated on for suspicion of carcinoma but received a final diagnosis of breast fibrosis. Those breast tissues were all found to be histopathologically inconspicuous.

NPY Receptor Autoradiography. Twenty-µm-thick cryostat sections of the tissue samples were processed for NPY receptor autoradiography as described in detail previously for other peptide receptors (27). One radioligand used was 125I-labeled PYY (2000 Ci/mmol; Anawa, Wangen, Switzerland), known to label NPY receptors specifically. For autoradiography, tissue sections were mounted on precoated microscope slides and stored at −20°C for at least 3 days to improve adhesion of the tissue to the slide. Sections were then processed according to Dumont et al. (28). They were first preincubated in 119 mM NaCl, 3.2 mM KCl, 1.19 mM KH2PO4, 1.19 mM MgSO4, 25 mM NaHCO3, 2.53 mM CaCl2 × 2H2O, and 10 mM D-glucose (pH 7.4) preincubation solution for 60 min at room temperature. The slides were then incubated in a solution containing the same medium as the preincubation solution in which the
following compounds were added: 0.1% BSA, 0.05% bacitracin (pH 7.4), and the radioligand at an approximate concentration of 22 pm 125I-labeled PYY. The slides were incubated at room temperature with the radioligand for 120 min. To estimate nonspecific binding, paired serial sections were incubated as described above, except that 25 nm PYY were added to the medium. To be able to distinguish Y1 from Y2 subtypes, displacement experiments were performed with 22 pm 125I-labeled PYY and increasing amounts of nonradioactive NPY, the Y1-selective ligand [Leu11, Pro34]-NPY, and the Y2-selective ligand PYY(3–36) to generate competitive inhibition curves on successive sections using the same incubation medium as above. Additional analogues used as competitors in these displacement experiments included PP and PYY(13–36).

Displacement curves were performed for all compounds in a series of neo-
plastic and non-neoplastic tissues and led us to conclude that a 25-nM concen-
tration of [Leu11, Pro34]-NPY or PYY(3–36) was adequate to evaluate their
rank order of potencies in a given tumor and, therefore, to distinguish Y1 from
Y2 subtype expression in that tumor tissue. On completion of the incubation,
the slides were rinsed four times for 5 min each in ice-cold preincubation
solution (pH 7.4). The slides were rinsed twice in ice-cold distilled water,
and then dried under a stream of cold air at 4°C, aspoled to 1H-Hyrophilms,
and exposed for 7 days in X-ray cassettes.

To further distinguish Y1 from Y2 receptors, all cases demonstrating binding with 125I-labeled PYY were evaluated with the Y1-selective radioligand 125I-
labeled [Leu11, Pro34]-PYY and with the Y2-selective 125I-labeled PYY(3–36)
(29, 30). The experiments were performed with 125I-labeled [Leu11, Pro34]-
PYY as radioligand and unlabeled PYY or [Leu11, Pro34]-PYY as competitors
or with 125I-labeled PYY(3–36) as radioligand and PYY or PYY(3–36) as
competitors. Identical experimental conditions as mentioned for 125I-
labeled PYY were used. The autoradiograms were quantified using a computer-
assisted image processing system, as described previously (27, 31). Tissue
standards for iodinated compounds (Amersham, Aylesbury, UK) were used
for this purpose. A tissue was defined as receptor-positive when the absorbance
measured in the total-binding section was at least twice that of the nonspecific-
binding section (in the presence of 25 nm PYY). In each experiments, we have
included as positive controls Y1-expressing tissue (rat cortex) and Y2-express-
ing tissues (stratum oriens and radium of the rat hippocampus; Ref. 28).

In Situ Hybridization Histochemistry. Y1 and Y2 receptor mRNAs were
identified in selected normal and tumoral breast tissue samples with in situ
hybridization histochemistry on cryostat sections, as described previously in
detail (32). Oligonucleotide probes complementary to nucleotides 493–529 or
580–589 (33) of the human Y1 receptor gene and to nucleotides 223–252 (33)
of the human Y2 receptor gene, or 1008–1052 (34) of the rat Y2 receptor
gene (that sequence having 96% homology to the corresponding human one),
were synthesized and purified on a 20% polyacrylamide-8M urea sequencing gel
(Microsynth, Balgach, Switzerland). They were labeled at the 3
32P
dpm/mg tissue (mean
-32P]dATP (>3000 Ci/mmol; NEN, Life Science Products, Boston, MA)
and terminal deoxynucleotidyl transferase (Boehringer, Mannheim, Germany)
to specific activities of 0.9–2.0 × 10

9 M ), and cells were incu-
bated for 48 h. Media were refreshed after 24 h and peptides added again after
12 and 36 h. Cell growth was measured by cell counting using a Coulter
Counter (Model ZB; Coulter Electronics, Hialeah, FL) and a hemocytometer
after the trypsinization and the dispersion of cells.

RESULTS

Table 1 summarizes the NPY receptor incidence in breast tissue in
the main group of 89 patients. They were expressed in a total of 76 of
89 breast carcinomas tested. These were found in 61 of 69 ductal
10% carcinomas (58 of 64 invasive and 3 of 5 in situ) and 10 of 12 lobular
carcinomas. Within the group of NPY receptor-positive invasive
ductal carcinomas, we identified 15 cases with concomitant in situ
ductal carcinoma lesions sufficiently large to evaluate their NPY
receptor status: 14 of these in situ carcinomas expressed NPY recep-
tors. Of the special types of invasive carcinomas, two of three mucin-
ous carcinomas, two of two medullary and one of one tubular
breast carcinoma were NPY receptor-positive. The 13 NPY receptor-nega-
tive cases consisted of 8 ductal carcinomas (6 invasive and 2 in situ),
2 lobular carcinomas, 1 mucinuous, and 2 apocrine carcinomas. For Y1
and Y2 subtype characterization, the two approaches used in the
present study, i.e., the use of the universal radioligand 125I-labeled
PYY and its displacement by unlabeled Y1 and Y2 subtype-selective
analogues (28) or the use of the two Y1- and Y2-selective radioligands
(30), gave congruent results: Y1 was the predominantly expressed
receptor subtype in NPY receptor-positive tumors, with 100% inci-
dence of this subtype in receptor-positive tumors, whereas Y2 was
found only in 24% of cases (Table 1). In 58 of 76 (76%) of the
receptor-positive tumors, Y1 was found to be the only (46 cases) or the
predominant (12 cases containing more than 90% of Y1 compared with
Y2) receptor subtype expressed; whereas in 24%, both Y1 and Y2
were highly expressed. In none of the tumors was Y2 found alone. In
general, a comparable pattern of Y1 and Y2 expression was seen in the
in situ and invasive part of a ductal carcinoma from a single patient.

Another important observation was that Y1 was much more often
distributed homogeneously within the tumor than was the Y2 receptor,
which was expressed focally, i.e., in restricted areas of the tumor only,
in 55% of the cases. Even in tumors concomitantly expressing Y1 and
Y2, we found no tumor area that expressed Y2 alone; Y2 was only
found in areas where Y1 was also expressed. In the majority of
receptor-positive tumors, the density of NPY receptors was high. The
mean density value for the 58 Y1-expressing tumors was 4946 ± 485
dpm/mg tissue (mean ± SE; n = 58). The mean density values for the 18 cases expressing Y1 and Y2 were the following: (a) 9754 ± 684
dpm/mg tissue (mean ± SE; n = 18) for Y1; and (b) 5681 ± 782
dpm/mg tissue (mean ± SE; n = 18) for Y2. It should be noted that,
in the latter group, the values for Y2 were based, in the majority of the
cases, on measurements in a very restricted area of the tumor where

Table 1. Incidence of NPY receptors Y1 and Y2 in human breast tissues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>NPY-R incidence</th>
<th>Differentiation by Y1 and/or Y2 expression</th>
<th>Cases with focal R distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast carcinomas</td>
<td>76/89 (85%)</td>
<td>Y1, tumor type: *51/76 (67%)</td>
<td>21/58 (36%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mixed Y1/Y2 tumor type:</td>
<td>3/18 (17%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>18/76 (24%)</td>
<td>Y2:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Y2 only:</td>
<td>3/18 (17%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Y2 only: 1/18 (5%)</td>
<td>Y2 only:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0/76 (0%)</td>
<td>0/18 (0%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1/18 (5%)</td>
<td>1/18 (5%)</td>
</tr>
<tr>
<td>Non-neoplastic breast (ducts and lobules)</td>
<td>45/45 (100%)</td>
<td>Y1 tumor type:</td>
<td>0/76 (0%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2/45 (4%)</td>
<td>0/18 (0%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0/45 (0%)</td>
<td>0/18 (0%)</td>
</tr>
</tbody>
</table>

* Detected with “universal” ligand 125I-labeled PYY.

* Detected with Y1-selective (125I-labeled [Leu11, Pro34]-PYY) or Y2-selective (125I-labeled PYY(3–36)) radioligands.

* Y1-type tumors were defined as those tumors expressing predominantly Y1 but not Y2. Forty-six cases had only Y1; the remaining 12 cases had a density of Y2 amounting to <10% of the Y1 density.
the receptors were focally expressed. Conversely, the Y₁ values were based, in most cases, on measurements in the whole tumor sample characterized by a homogenous receptor distribution. In an additional group of six patients listed in Table 2, from whom primary breast tumors could be obtained together with all lymph node metastases, we found that the six primaries as well as all of the metastases were expressing NPY receptors; Y₁ was present in all cases, Y₂ in a few cases only, both in primaries as well as in metastases (Table 2).

As reported in Table 1, NPY receptors can be detected in all tested normal breast tissues as well. In 42% of the cases, the Y₂ receptor is expressed alone, whereas in none of the tested breast tissues is the Y₁ receptor expressed alone. However, in the remaining breast tissues, Y₁ and Y₂ can be expressed concomitantly (58%). The density of Y₁ and Y₂ receptors in all investigated individual breast tissue samples is high and comparable with the density found in breast cancers. The mean density values in non-neoplastic breast are higher for Y₂ than for Y₁, with 7377 ± 497 dpm/mg tissue (mean ± SE; n = 45) for Y₂ and 3793 ± 500 dpm/mg tissue (mean ± SE; n = 26) for Y₁.

Fig. 1a shows a typical and representative example of the NPY receptor expression in a sample containing a breast carcinoma surrounded by normal breast tissue. The breast carcinoma expresses Y₁ receptors only, as shown by the labeling of the tumor by ¹²⁵I-labeled PYY and its displacement with [Leu³¹, Pro³⁴]-NPY but not by PYY(3–36). These results are confirmed further by the additional experiments using two other radioligands: the tumor is labeled by the Y₁-selective ¹²⁵I-labeled [Leu³¹, Pro³⁴]-PYY but not by the Y₂-selective ¹²⁵I-labeled PYY(3–36) (Fig. 1a). Conversely, in the same tissue sections, the surrounding breast expresses predominantly Y₂ receptors, as shown by the opposite rank order of potency of NPY analogues, i.e., the high affinity of labeled and unlabeled PYY(3–36) but the low affinity of [Leu³¹, Pro³⁴]-NPY and [Leu³¹, Pro³⁴]-PYY.

Table 2 Incidence and density of Y₁ and Y₂ subtypes in the primary tumor and in all lymph node metastases of six patients with breast cancer³

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Receptor density</th>
<th>No. of metastases</th>
<th>Receptor incidence/Mean density</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Y₁</td>
<td>Y₂</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case 1 (ductal Ca)</td>
<td>7200</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Case 2 (lobular Ca)</td>
<td>1398</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Case 3 (ductal Ca)</td>
<td>12262</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Case 4 (ductal Ca)</td>
<td>12542</td>
<td>6535</td>
<td>6</td>
</tr>
<tr>
<td>Case 5 (lobular Ca)</td>
<td>9797</td>
<td>2879</td>
<td>3</td>
</tr>
<tr>
<td>Case 6 (ductal Ca)</td>
<td>9445</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

³ Density is expressed as dpm/mg tissue (mean ± SE where n > 2).

Fig. 1, a, NPY receptors in breast carcinoma and adjacent normal breast. A, H&E-stained section showing tumor (Tu) and normal breast (arrowheads). Bar = 1 mm. B, autoradiogram showing total binding of the universal ligand ¹²⁵I-labeled PYY with strong labeling of tumor and breast representing Y₁ and Y₂. C, autoradiogram showing ¹²⁵I-labeled PYY binding in the presence of 25 nM of the Y₁-selective [Leu³¹, Pro³⁴]-NPY. Complete displacement of the radioligand is seen in tumor but not in breast, suggesting Y₁ in the tumor. D, autoradiogram showing ¹²⁵I-labeled PYY binding in the presence of 25 nM of the Y₁-selective PYY(3–36). Complete displacement is seen in breast but not in tumor, suggesting Y₂ in the breast. E, autoradiogram showing total binding of the Y₂-selective ligand ¹²⁵I-labeled [Leu³¹, Pro³⁴]-PYY (in the presence of 25 nM of the Y₁-selective PYY). The tumor strongly expresses Y₁; the breast tissue is not or only very weakly labeled. F, autoradiogram showing nonspecific binding of ¹²⁵I-labeled [Leu³¹, Pro³⁴]-PYY (in the presence of 25 nM of the Y₂-selective PYY). G, autoradiogram showing total binding of the Y₂-selective ligand ¹²⁵I-labeled PYY(3–36). The tumor is not labeled, but the adjacent breast tissue expresses Y₂. H, autoradiogram showing nonspecific binding of ¹²⁵I-labeled PYY(3–36) [in the presence of 25 nM of PYY(3–36)]. J, Competition curves showing Y₁ in human breast carcinoma (top), Y₂ in normal breast (midIDDLE), and Y₁ in vessels (bottom). Top and bottom graphs, high-affinity displacement of ¹²⁵I-labeled PYY by PYY, [Leu³¹, Pro³⁴]-NPY, and [Leu³¹, Pro³⁴]-PYY, but not by PYY(3–36). Somatostatin (SS-14) is inactive. Middle graph, high affinity displacement of ¹²⁵I-labeled PYY by PYY and PYY(3–36) but not by [Leu³¹, Pro³⁴]-PYY, and [Leu³¹, Pro³⁴]-NPY. Somatostatin (SS-14) is inactive.
finity in Y₂-expressing normal breast and was inactive in Y₁ tumors. Furthermore, PP, known to have high-affinity binding for Y₄ (22), displaced ¹²⁵I-labeled PYY with low affinity in breast and in breast tumors. In selected cases, ionic manipulations of the incubation solution were performed as an additional way to differentiate between Y₁ and Y₂ (35). In the presence of 5 mM Mn²⁺, the Y₁ could not be detected in the tumors anymore, whereas Y₂ remained present in the adjacent breast.

Fig. 2 shows a high magnification of Y₂-expressing lobules and one duct from normal breast tissue labeled with the Y₂-selective ¹²⁵I-labeled PYY(3–36). Note that not all elements are homogeneously labeled. In normal breast, we have often found few lobules and ducts that were either nonhomogeneously labeled or unlabeled next to a majority of labeled elements.

In situ hybridization for Y₁ and Y₂ mRNAs was performed in cases selected for their high expression of the respective receptor proteins. We could consistently show Y₁ mRNA in the 12 investigated Y₁-type of tumors. Furthermore, it was possible to detect Y₂ mRNA in isolated Y₂-expressing tubules of the normal breast. Fig. 3 illustrates Y₁ mRNA in a breast tumor and Y₂ mRNA in a tubule of the normal breast and compares it with Y₁ and Y₂ receptor autoradiography using Y₁- and Y₂-selective radioligands, respectively.

To search for a potential function of NPY in cancer, we have tested the effect of NPY in the Y₁-expressing SK-N-MC tumor cells. NPY (10⁻⁷ m) for 24 h was able to inhibit tumor cell growth by >40%, whereas the Y₂-selective NPY(3–36) was inactive (Fig. 4). This growth-inhibitory effect was dose-dependent. Moreover, it was particularly pronounced during the first 24 h of NPY treatment (Fig. 4).

DISCUSSION

This study is the first evidence that the neuropeptide NPY may play a role in human cancer. It is remarkable that a great majority, i.e., 85% of the patients with breast cancers, often have an high expression of NPY receptors in their cancers. In all cases, the NPY receptor subtype Y₁ is expressed, whereas Y₂ is only expressed in 24% of the cases, and, when it is expressed, it never represents the predominant subtype of the tumor and is never found alone. In the 24% of the cases with...
a mixed expression of Y1 and Y2, a much more focal, topographically restricted distribution can be recognized for Y3 than for Y1, emphasizing once more the predominance of Y3 in tumors. Both ductal and lobular breast cancers, of the in situ and invasive types, as well as all lymph node metastases, can express NPY receptors.

We also give here the first evidence that Y1 receptors may be of functional relevance in tumor cell proliferation. Indeed, the addition of \(10^{-7}\) m NPY for 24 h to cultures of Y2-expressing human SK-N-MC cells in the growing phase was able to inhibit the growth of these cells by >40%. This NPY effect seems to be a specifically Y1-mediated process for the following reasons: (a) the SK-N-MC cells express Y1 receptors selectively (36, 37); and (b) the Y2-selective NPY(3–36) was unable to induce this antiproliferative effect under the same conditions. We conclude that NPY has antiproliferative properties at Y1 receptors; the degree of NPY-induced antiproliferation seen in SK-N-MC cells is remarkable, and compares favorably with antiproliferative effects reported for other peptide hormones such as somatostatin (38), which has an established role in clinical oncology.

Among the numerous cloned NPY receptors, the Y1, Y2, Y4, and Y5 currently represent the only fully defined subtypes (22). There are several arguments showing that the subtypes detected in the tumors of the present study correspond to Y1 and Y2. Pharmacological evidence for Y1 expression in tumors are: (a) specific binding of \(^{125}\)I-labeled PYY that is fully displaced in the high affinity range by the Y1-selective [Leu\(^{31}\), Pro\(^{34}\)]-NPY but not by PYY(3–36), PYY(13–36), or PP, compounds known to have high affinity for Y2 or Y3 (22, 28); (b) selective binding of \(^{125}\)I-labeled [Leu\(^{31}\), Pro\(^{34}\)]-PYY in the same tissues (30); (c) subtype-selective ionic dependence, i.e., the inability to detect Y1 in the presence of Mn\(^{2+}\), although Y2 is identified under these conditions (35); and (d) Y1 mRNA detected by \(\textit{in situ}\) hybridization in the tumor tissues. Furthermore, with those techniques, we could confirm in humans the data from previous reports in animals showing Y1-expression in vessels (39, 40).

Evidence for Y2 expression in normal breast include: (a) specific binding of \(^{125}\)I-labeled PYY displaced by nanomolar concentrations of the Y2-selective PYY(3–36) or PYY(13–36), whereas PP and [Leu\(^{31}\), Pro\(^{34}\)]-NPY were not active; (b) selective labeling of the breast tissues by \(^{125}\)I-labeled [Leu\(^{31}\), Pro\(^{34}\)]-PYY in the same tissues (30); (c) subtype-selective ionic dependence, i.e., the inability to detect Y2 in the presence of Mn\(^{2+}\), although Y1 is identified under these conditions (35); and (d) Y2 mRNA detected in \(\textit{in situ}\) hybridization in the tumors. Nevertheless, with those techniques, we could confirm in humans the data from previous reports in animals showing Y2-expression in vessels (39, 40).

Another novel observation is that the ducts and lobules of the non-neoplastic breast tissue, representing the tissue from which breast tumors originate, also express NPY receptors. Although all investigated breast tissues express NPY receptors, it is remarkable, however, that in all tested cases, the subtype Y2, rather than Y1, is consistently and predominantly detected in both lobules and ducts; the subtype Y1 is only found in a minority of cases and only concomitantly with Y2. Y1 was never identified as the unique subtype present in a normal breast tissue.

The observation that neoplastic human breast tissue can express a different NPY receptor subtype than non-neoplastic breast strongly suggests an alteration of the NPY receptor subtype expression during the process of neoplastic transformation of the breast. The normal condition appears to be a predominant Y2 expression by the non-neoplastic breast. However, in addition, Y1 may be present in some of the histologically inconspicuous breast tissues; it is yet unknown whether this Y1 expression is found preferentially in breast tissues surrounding a growing tumor and whether it may reflect an early sign of neoplastic transformation. In the frankly neoplastic tissue of both lobular and ductal cancers, the NPY receptor found predominantly in all cases is the Y1 type, although Y2 may be present concomitantly in some of the cases in a focal distribution. Therefore, neoplastic transformation of breast tissue may induce a switch of expression from receptor subtype Y2 to Y1. This switch in receptor expression possibly may be related to the increasing dedifferentiation of the tissue. Indeed, it was reported recently that, in rat pheochromocytoma PC12 cells, the NGF-differentiated cells were expressing Y2 preferentially, whereas the undifferentiated PC12 cells contained Y1 (41). This differentiation-specific expression of subtypes may be a general characteristic of NPY receptors.

The present study points toward a potential functional role of Y1 receptors in cancer, because NPY can inhibit the growth of the Y2-expressing SK-N-MC cells in culture; the high density and high incidence of Y1 in breast cancers suggest that these neoplasms may represent an important target for NPY-related drugs. First, long-term treatment with Y1-selective analogues (23, 24) may be used to inhibit tumor proliferation. Second, one may think of targeting breast tumors and metastases with radionabeled Y1 analogues for diagnostic \(\textit{in vivo}\) scintigraphic tumor detection or for receptor-mediated radiotherapeutic treatment of these tumors, in analogy to \(\textit{in vivo}\) targeting of other peptide receptors (1, 10, 11). One may argue that the Y1 receptors present in approximately one-half of the normal breast could interfere \(\textit{in vivo}\) with the tumor-Y1 targeting; however, the labeling of a low number of Y1 ducts and lobules disseminated within the whole breast is likely to be negligible compared with the focal labeling of the whole tumor mass. Moreover, for radiotherapy, it may not necessarily be a disadvantage to target the Y1-expressing breast tissue, in the event that the Y1 expression in this tissue turns out to reflect an early stage of neoplastic transformation. Y1 targeting of breast tumors may be superior to somatostatin, VIP, or gastrin-releasing peptide receptor targeting, as these three receptors are either expressed in lower incidence and/or heterogeneously in breast tumors (somatostatin receptors; Ref. 42), or concomitantly in the normal and tumoral breast tissue (VIP and gastrin-releasing peptide receptors; Refs. 43 and 44). NPY should therefore be added to the list of small regulatory peptides relevant to cancer, and to breast cancer in particular. It may be attractive to combine Y1 radioligands with radionabeled VIP, gastrin-releasing peptide, and somatostatin analogues, as all four peptide families can have their receptors overexpressed in breast cancers (42–44). Such a cocktail of four peptide radioligands, given concomitantly, may substantially increase the diagnostic sensitivity of the scintigraphic procedure as well as the radiotherapeutic efficacy.

REFERENCES


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