Gastrin-releasing Peptide Receptors in the Human Prostate: Relation to Neoplastic Transformation

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ABSTRACT

Bombesin-like peptides such as gastrin-releasing peptide (GRP) have been shown to play a role in cancer as autocrine growth factors that stimulate tumor growth through specific receptors. To search for potential clinical indications for GRP analogues, it is important to identify human tumor types expressing sufficient amounts of the respective receptors. In the present study, we have evaluated the expression of GRP receptors in human nonneoplastic and neoplastic prostate tissues using in vitro receptor autoradiography on tissue sections with 125I-Tyr4-bombesin as radioligand. GRP receptors were detected, often in high density, in 30 of 30 invasive prostatic carcinomas and also in 26 of 26 cases of prostatic intraepithelial proliferative lesions, corresponding mostly to prostatic intraepithelial neoplasias. Well-differentiated carcinomas had a higher receptor density than poorly differentiated ones. Bone metastases of androgen-independent prostate cancers were GRP receptor-positive in 4 of 7 cases. Conversely, GRP receptors were identified in only a few hyperplastic prostates and were localized in very low density in glandular tissue and, focally, in some stromal tissue. In all of the cases, the receptors corresponded to the GRP receptor subtype of bombesin receptors, having high affinity for GRP and bombesin and lower affinity for neuromedin B. These data demonstrate a massive GRP receptor overexpression in prostate tissues that are neoplastically transformed or, like prostatic intraepithelial neoplasias, are in the process of malignant transformation. GRP receptors may be markers for early molecular events in prostate carcinogenesis and useful in differentiating prostate hyperplasia from prostate neoplasia. Such data may not only be of biological significance but may also provide a molecular basis for potential clinical applications such as GRP-receptor scintigraphy for early tumor diagnosis, radiotherapy with radiolabeled bombesin-like peptide analogues, and chemotherapy with cytotoxic bombesin analogues.

INTRODUCTION

Receptors for regulatory peptides have been shown over the last 15 years to be overexpressed in several types of human neoplasia. Somatostatin receptors are found in large quantities in most neuroendocrine tumors (1), vasoactive intestinal peptide receptors are expressed in most epithelial tumors (2), and cholecystokinin-B receptors are identified in medullary thyroid carcinomas and small cell lung cancers (3, 4). These in vitro observations have led to the development of diagnostic and radiotherapeutic applications, using radiolabeled peptides for in vivo receptor scintigraphy (5–7) or peptide radiotherapy (8) in tumor patients. Furthermore, peptides linked to cytotoxic drugs (9) or stable peptide agonists or antagonists (10) have been used for long-term targeted chemotherapy in animal tumor models.

GRP2 belongs to the family of bombesin-like peptides that includes the amphibian peptide bombesin as well as the mammalian counterparts GRP and neuromedin B (11). These peptides have been shown to play a role in various tumor models and in human cancer. For instance, they were shown by the group of Rozengurt et al. (12) to be mitogenic in Swiss 3T3 cells. Moreover, Cutitta et al. (13) showed that bombesin and GRP can stimulate small cell lung cancer growth and that this action is part of an autocrine feedback mechanism involving the expression of these peptides as well as their receptors in the tumor cells (14, 15). More recently, GRP and bombesin were shown to play a possible role in prostate cancers as well. For instance, it was shown that GRP can stimulate cell proliferation in the androgen-independent human prostatic carcinoma cell line PC3 and that antagonists of the GRP receptor inhibit the growth of a number of prostatic carcinoma models, either cancer cells in vitro or xenografts in syngeneic rats or nude mice (16–19).

GRP mediates its action through membrane-bound receptors. These receptors correspond to one of the subtypes of bombesin-like peptide receptors, namely the GRP receptor, which is characterized by a high-affinity binding for GRP and bombesin and only a moderate affinity for neuromedin B. These receptors are members of the large superfamily of G-protein-coupled receptors with seven transmembrane domains. Bombesin-like peptide receptors seem to mediate the mitogenic action of bombesin-like peptides in neoplasias; therefore, the information about the presence of bombesin-like peptide receptors, in particular of GRP receptors, is crucial for the evaluation of the bombesin-like peptide action in tumors. GRP receptors have been detected in various types of tumor cell lines (20–23). To predict the potential implications of bombesin-like peptides in patients with prostatic cancers, it is mandatory to know the incidence and the density of GRP receptors in human tumor tissues. For such an evaluation, the method of choice is in vitro receptor autoradiography (24). It allows the localization of peptide receptors in complex tumor samples obtained after surgical resection. In addition to prostatic carcinomas, such tissues usually contain normal prostate, hyperplastic prostate, and often PIN (25). This morphological method, therefore, allows us to distinguish the receptor expression in the nonneoplastic prostate and its malignant counterparts in individual tissue samples.

The aim of the present study was the evaluation of the incidence and the density of GRP receptors in a large number of human prostatic samples containing normal prostate and various stages of prostatic neoplastic transformation. The method used was in vitro receptor autoradiography on tissue sections, using iodinated [Tyr4]-bombesin as radioligand.

MATERIALS AND METHODS

Patient Tissues. Prostate tissue samples (peripheral zone) were obtained from 50 patients, ages 52–86 years, with primary prostate (36 cases) or primary bladder (14 cases) cancer operated on at the Department of Urology, University Hospital (Berne, Switzerland). In the 14 patients with bladder cancer, we studied only the prostate tissue; 4 of them had prostate cancer in addition to bladder cancer. Several large samples of tissues were collected from the patients and kept frozen at −80°C for up to 1 year. The tumor characteristics are listed in Table 1. In addition, seven samples of prostate cancer, metastatic to the bones and refractory to hormone treatment (androgen-independent), obtained previously from the University of Uppsala (26), were investigated. The diagnosis and grading were reviewed and formulated by use of cryostat as well as paraffin-embedded sections, according to the guidelines issued by Mostofi (WHO; Ref. 27). All of the tumor samples were evaluated...
for their Gleason scores (28). The high-grade PIN stage was also evaluated in cryostat sections and defined as atypical prostatic intraepithelial proliferation containing basal cells identified immunohistochemically by the presence of high molecular weight cytokeratins (i.e., 34βE12 antibody) to make a distinction from invasive cribriform carcinomas (29, 30).

**GRP Receptor Autoradiography.** Twenty-μm thick cryostat sections of the tissue samples were processed for GRP receptor autoradiography as described in detail previously for other peptide receptors (31). The radioligand used was 125I-Tyr4-bombesin, known to specifically label GRP receptors (32). For autoradiography, tissue sections were mounted on precleaned 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 Vigna (33). Slides were then dried at 4°C under a stream of cold air. The slides were placed in 10 mM HEPES buffer (pH 7.4) for 5 min at room temperature. They were then incubated in 10 mM HEPES, 130 mM NaCl, 4.7 mM KCl, 5 mM MgCl2, 1 mM ethyleneglycol-bis (β-aminoethylether)-N,N′-N′-tetraacetic acid, 0.1% BSA, 100 μg/ml bacitracin (pH 7.4), and approximately 100 pm 125I-Tyr4-bombesin-14 (2000 Ci/mmole; Anaqua, Wangen, Switzerland) in the presence or absence of 1 μM bombesin for 1 h at room temperature. Additional sections were incubated in the presence of increasing amounts of nonradioactive bombesin, GRP, neuromedin B, or somatostatin to generate competitive inhibition curves. After incubation, the sections were washed four times for 2 min each in 10 mM HEPES with 0.1% BSA (pH 7.4) at 4°C. Finally, the slides were rinsed twice for 5 s each at 4°C in distilled water. The slides were then dried at 4°C under a stream of cold air. The slides were placed in apposition to [1H]Hyperfilms (Amersham, Little Chalfont, United Kingdom) and exposed for 7 days in X-ray cassettes.

The autoradiograms were quantified using a computer-assisted image processing system, as described previously (31). Tissue standards for iodinated compounds (Amersham) 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 10⁻⁶ M bombesin).

**RESULTS**

Table 1 summarizes the GRP receptor status in prostate tissues. The data obtained by quantitative receptor autoradiography from the resected prostate samples of all 36 of the prostate cancer and the 14 bladder cancer patients are listed. Four types of tissues were investigated: (a) invasive prostatic carcinoma; (b) high-grade PIN; (c) prostatic hyperplasia; and (d) prostatic stroma; however, not all of the samples contained invasive prostatic carcinoma and/or high-grade PIN tissue. In a few samples resected at the border of the carcinoma and devoid of neoplastic tissue, GRP receptors were quantified only in the stroma and hyperplastic glands.

Table 1 shows that neoplastic prostatic tissue expresses GRP receptors very frequently:

(a) All of the 30 cases with primary invasive prostatic carcinomas were found to express GRP receptors. In the majority (83%) of the cases, a high to very high density of receptors (>1000 dpm/mg tissue) was found (Table 1, No. 1–21 and 37–40); in five cases (No. 12, 16, 19, 21, and 22) a heterogeneous distribution of the receptors within adjacent tumor regions was found. The only three available cases with high (i.e., 7–9) Gleason scores (No. 21, 24, and 26) were all associated with a very low density of GRP receptors. One case with the lowest levels of GRP receptors was identified as G3 grade with a Gleason score of 9; this patient did not have elevated levels of prostate-specific antigen in the serum (below 4 ng/ml), suggestive of a particularly poor differentiation or dedifferentiation of the tumor.

(b) All of the 26 tissue samples containing an area of PIN were GRP receptor-positive. All but one case (No. 45) had high to very high densities of GRP receptors. The PIN area quantified for GRP receptors consisted primarily of high-grade PIN, in particular PIN III, as a clearly identifiable stage (25). PIN I, which is extremely difficult to distinguish from hyperplasia in cryostat sections (25), may have been present in some of the cases but was not evaluated separately. In several cases, a heterogeneous distribution of GRP receptors was observed, with some PIN devoid of receptors located next to PIN with high receptor values (i.e., No. 44), possibly as a reflection of the different PIN stages (I, II, III) present in these samples.

In addition, Table 1 shows that nonneoplastic prostatic tissue only rarely expressed a measurable amount of GRP receptors, and normal prostatic glandular epithelium did not express GRP receptors; prostatic hyperplasia expressed GRP receptors in 23 of 50 cases; however, in all but two of these cases (No. 4 and 27), the density of the receptors was very low, <500 dpm/mg tissue. The prostatic stroma was GRP receptor-positive in 16 of 50 cases; however, in these positive cases, the prostatic stroma was only focally GRP receptor-positive, i.e., receptors were found in a restricted area of the whole prostatic stromal tissue; stromal values in Table 1 represent GRP receptor densities measured in stromal sites having the highest labeling found within a sample. Except for three cases (No. 13, 39, 44), the peak receptor density values obtained under these measurement conditions were below 1500 dpm/mg tissue.

Among the seven bone metastases of androgen-independent prostatic cancers [all of them of poor differentiation (G3)], four cases were GRP receptor-positive (respective values: 9402, 1226, 4800, and 600 dpm/mg tissue).

A comparison of the GRP receptor expression in malignant neo- plastic prostatic tissue versus nonneoplastic prostatic tissue shows, as seen in Table 1, that the receptor incidence is much higher in carcinoma tissue and high-grade PIN as compared with nodular hyperplastic prostate and stroma; also the receptor density, calculated as the mean of all of the values (Table 2), is much higher in prostate carcinoma and high-grade PIN as compared with prostatic hyperplasia. In every single case, the receptor density is usually considerably higher in the neoplastic GRP receptor-positive tissues than in the surrounding nonneoplastic GRP receptor-positive tissues.

Fig. 1 illustrates an example of a strongly GRP receptor-positive invasive prostatic carcinoma adjacent to a receptor-negative prostatic hyperplasia. Fig. 2, A–C, illustrates a case consisting of an area of GRP receptor-positive high-grade PIN surrounded by receptor-negative normal prostate gland. Fig. 2 (D–F) demonstrates high densities of GRP receptor expression in a high-grade PIN area with one adjacent PIN devoid of GRP receptors. Here, PIN is defined by the presence of basal cells positively immunostained for high molecular weight cytokeratins. Fig. 3 shows a GRP receptor-positive bone metastasis from an androgen-independent invasive prostatic carcinoma. Fig. 4 is an example of a focal area of GRP receptor-positive prostatic stroma surrounded by receptor-negative prostatic hyperplasia as well as additional receptor-negative stromal tissue.

The receptor subtype identified in the prostatic invasive carcinoma and PIN corresponded to the GRP receptor subtype of the bombesin-like peptide receptor family; as shown in Fig. 5, there was high-affinity binding for GRP and bombesin and lower affinity for neuromedin B. Unrelated peptides like somatostatin were inactive. The same receptor subtype was also identified in the bone metastasis (Fig. 5) and in the prostatic stromal tissue (data not shown).

**DISCUSSION**

This is the first extensive evaluation of GRP receptors in primary human prostatic tumors using morphological methods such as receptor autoradiography. The main finding is a high incidence and a high density of GRP receptors not only in invasive prostatic carcinomas but also, most interestingly, in the earliest phase of neoplastic transformation of the prostate, namely the PIN (25). This is in contrast to the absence or, at best, the low incidence and the low density of GRP receptors in nonneoplastic prostatic tissue, in particular in benign...
hyperplastic prostate. Such data suggest that GRP receptor expression is directly and strongly related to the neoplastic condition of the prostate with the most remarkable shift of expression of GRP receptors observed between the hyperplastic stage and the PIN stage. This study cannot establish with sufficient precision at which of the three PIN stages the overexpression of receptors begins. Indeed, the PIN stage detected in this study is primarily PIN III, which is clearly identifiable as such on cryostat-sectioned material. Low-grade PIN, because it is histologically difficult to identify in cryostat sections, has not been assessed separately and, therefore, no information is available on the receptor status of low-grade PIN.

<table>
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<th>No.</th>
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### Footnotes

a GRP-R, GPR receptor(s); *, high-grade PIN not identified in that sample; ht, heterogeneous receptor distribution; —, corresponding tissue not present.
b In these patients, only the prostate tissue was investigated. In 4/14 patients, prostate cancer was found incidentally.
prostatic carcinoma. These lesions are highly associated with coex-
istent prostatic cancers and have similar DNA ploidy, antigenic com-
position, and kinetic potentialities (25). It is recognized that detailed
knowledge of PIN may have important impact on future patient care;
therefore, a marker of this histological entity, such as GRP receptors,
may be of considerable clinical interest.

The prostatic carcinomas examined in this study were mainly of a
differentiated grade (G1 and G2 with Gleason scores of 3–6). There
were only three poorly differentiated (G3 with Gleason scores of 7–9)
prostatic carcinomas included in this study. Despite the uneven rep-
resentation of the three differentiation grades G1, G2, and G3, it may
be suggested, based on the low GRP receptor level in the few poorly
differentiated prostatic carcinomas, that undifferentiated primary
prostate carcinomas may not express or perhaps may have lost some
of their GRP receptors. It is unclear yet whether the same general
conclusion is valid for the androgen-independent bone metastases
because they can sometimes express high levels of GRP receptors
even though they are poorly differentiated. More G3 cases, both
primary tumors and metastases, will be necessary for a more detailed
evaluation of this aspect.

In a few cases a heterogeneous distribution of receptors was ob-
erved in histologically homogenous tumor areas. Heterogeneous
receptor distribution was more often seen in tumors with a lower
number of GRP receptors, reflecting possibly the presence of poorly
differentiated tumor regions. It is also possible that the observed
receptor heterogeneity is due to the presence of different tumor clones.

Several subtypes of the bombesin-like peptide receptors have been
described: the neuromedin B receptor with high affinity for neurome-
din B, the GRP receptor with high affinity for GRP but lower affinity
for neuromedin B (33, 34), and the bombesin 3 receptor subtype, an
orphan receptor for which no endogenous ligand has yet been found
(35). The pharmacological characterization of the receptor subtype in
malignant neoplastic prostate tissues, as identified in the present
study, points clearly toward the GRP receptor subtype, with very high
affinity for GRP and bombesin and lower affinity for neuromedin B.
This is in agreement with a recent in situ hybridization study evalu-
ating the mRNAs for the three bombesin-like peptide receptor sub-
types in prostatic tissues: only the GRP receptor subtype mRNA was
detected both in nonmalignant and malignant prostate tissues (36).
The mRNA staining in cancerous tissue ranged widely from very
intense to not detectable, whereas nonmalignant tissue displayed a low
level of message (36). These data may point toward an up-regulation
of the GRP receptor expression in prostate neoplasia; gene up-regu-
lations, for instance of the COSVIc gene, have recently been identified
in prostate cancer, for which they may have diagnostic value (37).

Although nonneoplastic prostatic tissues such as glandular hyper-
plasia and stroma were GRP receptor-negative in the majority of the
cases; a few patients showed prostatic tissue or parts of prostatic tissue
being weakly GRP receptor-positive. In those cases, the receptor
density was usually several times lower in the nonneoplastic than in
the neoplastic tissues. One possible explanation for the expression of
a low density of receptors in prostatic hyperplasia may be the presence
of an early phase of malignancy not readily detectable histologically
in our cryostat sections. Alternatively, the GRP receptor expression
seen in nonneoplastic prostate tissue in this study may be related to
neuroendocrine elements known to be present in the normal prostate.
Indeed, bombesin-like peptides and their receptors have been consid-
ered as markers of prostatic neuroendocrine tissues (38, 39). The very

Table 2  Density of GRP receptors in prostatic hyperplasias, PIN, invasive prostatic
carcinomas, and bone metastases of prostate cancer

<table>
<thead>
<tr>
<th>GRP receptor-positive tissues</th>
<th>GRP receptor density [dpm/mg tissue (mean ± SE)]</th>
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<tbody>
<tr>
<td>Hyperplasias (n = 23)$^a$</td>
<td>201 ± 31$^a$</td>
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<tr>
<td>High-grade PIN (n = 26)</td>
<td>4351 ± 649</td>
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<tr>
<td>Invasive carcinomas (n = 30)</td>
<td>5241 ± 927</td>
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<tr>
<td>Bone metastases (n = 4)$^a$</td>
<td>3863 ± 2018$^a$</td>
</tr>
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$^a$ The other tissue samples with GRP receptor-negative hyperplasia (n = 26) or bone
metastases (n = 3) are not included.

Fig. 1. High density of GRP receptors in an invasive prostatic carcinoma but not in the adjacent pro-
static hyperplasia. A, H&E-stained section. Ca, carcinoma; ph, prostate hyperplasia. Bar, 1 mm. B,
autoradiogram showing total binding of $^{125}$I-Tyr$^4$-
bombesin. Strong labeling of the carcinoma but not of
the hyperplasia is seen. C, autoradiogram showing
nonspecific binding of $^{125}$I-Tyr$^4$-bombesin (in
the presence of 10$^6$ M bombesin). D–E, details of stained
section (A) at high magnification; D, carcinoma; E,
hyperplasia. Bar, 0.1 mm.
Fig. 2. GRP receptors in high-grade PIN. A–C, high density of GRP receptors in a high-grade PIN area but not in the surrounding normal prostate gland. A, H&E-stained section. P, prostate gland. Bar, 1 mm. B, autoradiogram showing total binding of $^{125}\text{I}-\text{Tyr}^4$-bombesin. Strong and selective labeling of high-grade PIN is seen. Normal prostate is negative. C, autoradiogram showing nonspecific binding of $^{125}\text{I}-\text{Tyr}^4$-bombesin. D–F, nonhomogeneous distribution of GRP receptors in a high-grade PIN area. D, section immunostained for high molecular weight cytokeratins showing basal cells (arrowhead) in PIN and normal prostate gland (P). Bar, 1 mm. E, autoradiogram showing total binding of $^{125}\text{I}-\text{Tyr}^4$-bombesin. All of the PIN areas except one (*) are labeled. F, autoradiogram showing nonspecific binding. Insert, basal cell staining (arrowhead) for high molecular weight cytokeratins of the section seen in D at higher magnification. Bar, 0.01 mm.
low level of receptors in hyperplastic glands could, therefore, represent GRP receptors located in the normal neuroendocrine cells of the glands (38, 39).

The observation of GRP receptors located in prostatic stroma is intriguing. The relative low incidence of cases with stromal GRP receptors as well as the focal distribution of these receptors suggest that it is not a general phenomenon, as compared for instance with the ubiquitous expression of somatostatin receptors in the smooth muscle cells of most of the prostatic tissue (40). The stromal GRP receptor expression may be simply a local reaction to cancer; alternatively, it could be part of the physiological stromal-epithelial interactions, known to play a role in the prostate development (40, 41).

Even if the exact biological role of GRP receptors in the normal and neoplastic prostate is not yet established, the present in vitro receptor data have a number of important therapeutic and diagnostic implications. The very high GRP receptor density in prostatic invasive carcinomas and high-grade PIN suggests that prostate neoplasia represents an adequate type of cancer to perform GRP-related early clinical trials:

(a) Over the last several years, potent and selective bombesin antagonists have been developed (17, 34, 42). Several of these antagonists are compounds which have been shown in various animal tumor models to strongly inhibit tumor growth (17, 43). Other bombesin analogues have been designed as carriers for the radicals doxorubicin and 2-pyrrolinodoxorubicin to create hybrid cytotoxic drugs (44); they were also shown to inhibit the growth of cancer cell lines, including PC3 human prostate cancer cells (44). Therefore, the application of these types of compounds as long-term treatment of human prostate cancer may be considered as a future goal.

Fig. 3. GRP receptors in a bone metastasis of an androgen-independent prostatic carcinoma. A, H&E stained section. Ca, carcinoma; b, bone. Bar, 1 mm. B, autoradiogram showing total binding of 125I-Tyr4-bombesin. C, autoradiogram showing nonspecific binding.

Fig. 4. Focal expression of GRP receptors in a stromal area of a prostatic cancer sample. A, H&E-stained sections. *, stroma. ph, prostate hyperplasia. Bar, 1 mm. B, autoradiogram showing total binding of 125I-Tyr4-bombesin. Only a restricted area of the stroma is labeled (white star). Other stromal areas (black star), as well as prostate hyperplasia (ph), are negative. Several nonspecifically labeled prostate stones (arrowhead) are visible. C, autoradiogram showing nonspecific binding.
(c) As a logical consequence and further development, a radiothera-
dapy comparable to that using 90Yttrium-labeled octreotide (8), aiming
at the destruction of GRP receptor-positive tumors, could possibly be
envisioned using adequate bombesin analogues linked to β emitters
such as Rhenium-labeled bombesin analogues (45–47). Not only the
prostate invasive carcinomas but also that of early neoplastic
phases, such as high-grade PIN, would be of great potential interest.

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