**Upregulation of β3-Adrenergic Receptor mRNA in Human Colon Cancer: A Preliminary Study**

Maria Grazia Perrone\(^a\)  Maria Notarnicola\(^b\)  Maria Gabriella Caruso\(^b\)  Valeria Tutino\(^b\)  Antonio Scilimati\(^a\)

\(^a\)Dipartimento Farmaco-Chimico, Università di Bari, Bari, and \(^b\)Laboratory of Biochemistry, National Institute of Digestive Disease, Castellana Grotte, Italy

---

**Key Words**

β-Adrenergic receptor · Cell proliferation · Human colon cancer

---

**Abstract**

**Objective:** Tumor cell proliferation and migration, as well as metastasis formation, can be affected by several neurotransmitters, which therefore seem to be involved in the most important aspects of the malignant phenotype. In particular, modified β-adrenergic functions seem to be associated with proliferative alterations of numerous cancer cell lines. Pharmacological modulation of β-adrenoceptors (β-ARs) affects tumor cell growth in several experimental systems, and inhibition of metastasis formation by β-AR antagonists in in vivo models has recently been reported. Initial epidemiological studies have provided evidence that β-blockers can reduce cancer incidence, thus suggesting a possible role also in cancer prevention. 

**Methods:** Colorectal mucosa and cancer tissue were obtained from 41 patients. Specimens were taken within 1 h after the surgical procedure and stored at −80°C until assayed. The gene expression of β1-, β2- and β3-ARs in cancer tissue and normal surrounding mucosa was measured by real-time PCRs.

**Results:** Comparable levels of β1- and β2-AR mRNA were found to be expressed in normal mucosa and cancer tissues. A significant difference in β3-AR mRNA levels between normal mucosa and cancer tissues was found, with β3-AR mRNA expression being twice as high in cancer tissue than normal mucosa.

**Conclusion:** The results of the present study indicate that β3-AR mRNA is up-regulated in human colon cancer, thus suggesting the possible involvement of β3-AR in malignant transformation in the human colon.
Upregulation of β3-Adrenergic Receptor in Colon Cancer

seem to be involved in the most important aspects of the malignant phenotype. In particular, modified β-adrenergic functions associated with proliferative alterations of numerous cancer cell lines have been identified [3].

From a mechanistic point of view, it has been shown that biochemical pathways activated by β-adrenoceptors (β-ARs) cross-talk extensively with protein-tyrosine-kinase-activated pathways [4], as well as with cyclic-adenosine-monophosphate (cAMP)-linked pathways, thus likely affecting the proliferation, migration and metabolic behavior of cancer cells. Accordingly, pharmacological modulation of β-ARs affects tumor cell growth in several experimental systems, and inhibition of metastasis formation by β-AR antagonists in vivo models has recently been proved [5].

Importantly, initial epidemiological studies have provided evidence that β-blockers can reduce cancer incidence [5], thus suggesting a possible role also in cancer prevention.

β-ARs are classified into β1, β2 and β3 subtypes. Of particular relevance, β2-AR expression has been found in human breast, prostate and colon cancer tissues (table 1), and it has also been demonstrated that β2-AR signaling is involved in cell migration and metastasis formation in vitro and in vivo systems [5]. The level of expression and the role of β3-AR in cancer tissue have never been investigated. The β3-AR, like the other β-ARs, is a 7 transmembrane domain G protein-coupled receptor. It is usually coupled to a Gs protein, and its stimulation increases the production of cAMP [6, pp. 11–14]. β3-AR is expressed in several tissues (table 1), such as adipose tissue [7], heart [8], uterus [9], bladder [10] and colon [11], and modulates different physiological functions. Due to its wide tissue distribution, it can be considered a reasonable drug target for the treatment of several pathologies [12]. β3-AR is commonly known as a target to treat obesity and type 2 diabetes. Recently, the interest around this β-AR subtype increased markedly due to findings of its involvement also in heart failure and overactive bladder [8, 10].

It is also known that the β1- and β2-ARs are crucially involved in the onset, metastasis formation and progression of several types of cancers [13]. Herein, we report a preliminary exploration of the changes in expression of the β3-AR in human colon cancer as well as normal colon tissue.

The Three β-AR Subtypes

β-ARs belong to the G protein-coupled receptors, which are characterized by 7 transmembrane domains and 3 intracellular and 3 extracellular loops (fig. 1).

The N-terminus of all 3 β-ARs is extracellular and glycosylated. The C-terminus is intracellular, but unlike the β1- and β2-ARs, β3-AR has no sites for phosphorylation by protein kinase A (PKA) and β-AR kinase. β3-AR differs from the classical β1- and β2-ARs with respect to its regulatory properties (table 1).
It is known that desensitization of $\beta_1$- and $\beta_2$-AR responses upon agonist stimulation involves the phosphorylation of occupied receptors, uncoupling and internalization [14]. Both $\beta_1$- and $\beta_2$-ARs have serine and threonine residues in the C terminus tail that act as substrates for G protein-coupled receptor kinases, as well as consensus sequences for phosphorylation by cAMP-dependent protein kinase (PKA).

The $\beta_3$-AR lacks a PKA phosphorylation site and has fewer serine and threonine residues in the C-terminus tail. It has been shown that the $\beta_3$-AR is resistant to short-term agonist-promoted desensitization [15]. Studies on chimeric $\beta_2$/$\beta_3$-ARs showed that domains within the C-terminus tail and in the second and third intracellular loops of the $\beta_2$-AR were the major determinants of desensitization [15, 16]. Thus, for example, in human myometrium, $\beta_2$-AR undergoes functional desensitization after long-term exposure to salbutamol, a $\beta_2$-AR agonist, which is associated with a significant reduction in the number of $\beta_2$-AR binding sites. In contrast, sustained stimulation of the $\beta_3$-AR did not modify its subsequent functional effects, and its binding sites also remained unchanged after such a treatment [17]. Thus, these data suggest that following prolonged activation by the sympathetic nervous system, the $\beta_3$-AR response may be preserved, while the $\beta_1$- and $\beta_2$-AR responses are diminished.

### Materials and Methods

#### Patients

After obtaining informed consent, 41 patients with colorectal cancer (21 males and 20 females, mean age 66.1 ± 2.2 years) were consecutively enrolled in the study. Colorectal mucosa and cancer tissue were obtained from each of them. Specimens were taken within 1 h after the surgical procedure and stored at −80 °C until assayed.

Clinical characteristics and histopathological features were recorded for each patient (table 2).

**$\beta_1$, $\beta_2$- and $\beta_3$-AR Gene Expression Analysis**

Total RNA from normal colorectal mucosa and cancer tissues was isolated by TRI reagent (Molecular Research Centre Inc., Cincinnati, Ohio, USA) according to the manufacturer’s instructions. Briefly, the tissue was homogenized in 0.25 ml of cold 0.9% NaCl. Then, 0.75 ml of TRI reagent and 0.2 ml of chloroform were added to the homogenate. The samples were shaken vigorously and centrifuged. The RNA present in the aqueous phase was precipitated with 0.5 ml of isopropanol. The RNA pellet was washed once with 1 ml of 75% ethanol, dried, resuspended in sterile water and quantified by UV absorbance. Two micrograms of total RNA were used for the reverse transcription reaction, which was performed in a final volume of 20 μl at 41°C for 60 min, using 30 pmol of antisense primer (table 3) for analysis of the $\beta_1$, $\beta_2$- and $\beta_3$-ARs and the β-actin gene. The human β-actin gene was used as an internal control, chosen as a reference gene because it is a housekeeping gene.

Real-time PCRs were performed in a final volume of 25 μl containing 2 μl of cDNA, master mix with SYBR Green (iQ SYBR Green Supermix, Bio-Rad, Milan, Italy) and sense and antisense
We studied the gene expression of $\beta_1$, $\beta_2$, and $\beta_3$-ARs in colorectal cancer and normal surrounding mucosa from 41 patients operated for colorectal carcinoma. The clinical and histopathological features of all patients are given in Table 2. We were able to detect $\beta_1$, $\beta_2$, and $\beta_3$-AR mRNA in human colon mucosa and cancer tissue from all patients examined. $\beta_3$-AR mRNA was expressed at higher levels than $\beta_1$- and $\beta_2$-AR mRNA in normal mucosa as well as cancer tissue, and the difference in expression levels was statistically significant ($p < 0.05$).

Interestingly, a statistically significant difference was found between levels of $\beta_3$-AR mRNA in cancer tissue compared with normal mucosa (1.88 ± 0.24 vs. 1.16 ± 0.14, mean value ± SEM, $p = 0.036$; Table 4). An increase in $\beta_3$-AR mRNA levels in cancer tissue was observed in 32 of 41 patients (78%).

No difference was detected in $\beta$-AR mRNA levels with regard to age, sex, tumor site, stage of disease and histological differentiation (data not shown).

### Discussion

This study provides the first evidence of the presence of altered $\beta$-AR gene expression in human colorectal cancer. In particular, we found higher $\beta_3$-AR mRNA levels in cancer tissue than in normal mucosa, whereas the $\beta_1$- and $\beta_2$-AR subtypes were expressed at comparable levels in normal mucosa and cancer tissues.

Up to now, little has been done in quantifying the $\beta$-AR subtypes in human gastrointestinal tissue with the aim of elucidating the role of these receptors in physiopathological conditions.

Immunohistochemical studies have localized the $\beta_3$-AR in vascular and nonvascular smooth muscle of the human gastrointestinal tract, supporting a role for this receptor in the control of blood flow and motility in the human gastrointestinal tract [18]. Functional and molecular evidence for $\beta$-ARs in human colonic muscle and mucosa has been reported [11]. The $\beta_1$-AR appears to be involved in the relaxation of the thin longitudinal smooth muscle of the human colon, whereas the $\beta_3$-AR seems to play a role in relaxation of the taenia coli. In fact, although the presence of $\beta_3$-AR mRNA was previously demonstrated in mucosal samples from rat colon, Roberts et al. [11] found no evidence for $\beta_3$-AR mRNA in human colonic mucosa. This observation is different from the results of our present study, which demonstrate not only the presence of $\beta_3$-AR mRNA in mucosal samples from human colon but also evidence of its overexpression with respect to the other AR subtypes. This discordance is likely attributable to the different methodological procedures used to analyze gene expression of the receptor.

Moreover, we found a significant upregulation of $\beta_3$-AR expression in cancer tissue compared to normal mucosa.
It is known that the β-AR system plays an important role in both the regulation of growth and activation of lipid mobilization in fat cells. Modulation of lipid metabolism has also been considered a potential approach to the treatment of obesity and the metabolic syndrome [19]. Recent epidemiological studies have shown an association between obesity and functional bowel disorders [20–22], pointing to obesity as a risk factor for colorectal cancer due to chronic inflammation of colon tissue [23]. In addition, experimental evidence has demonstrated the association of polymorphisms in the β2- and β3-AR genes with obesity and insulin resistance [24], as well as with a greater susceptibility to colon cancer in obese subjects [25]. These observations are in agreement with the idea that β-ARs are involved in neoplastic transformation of colon tissue, and our results underline a possible functional role of β3-AR in colon cancer.

Further studies are in progress aimed at (1) proving the involvement of β3-AR in malignant transformation in the human colon, (2) investigating the impact of polymorphisms of β-ARs on gene expression in cancer tissues and normal mucosa, (3) identifying the factors influencing the upregulation of β3-AR in cancer tissues compared to normal tissues and (4) identifying potential β3-AR antagonists and inverse agonists as anticancer drugs.

Acknowledgements

The authors thank the University of Bari for the financial support, the Italian Ministero della Salute for a research grant (No. ICS-160.2/RF99.65) and Miss Benedetta D’Attoma for her excellent technical assistance.

Table 3. Sequences of amplification primers of β1-, β2- and β3-ARs

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>β1-AR</td>
<td>sense 5’-CTGTCTCAGCAGTGGACAGCGG-3’</td>
</tr>
<tr>
<td></td>
<td>antisense 5’-CAGCAGGCTCTGTTAGCGGAAG-3’</td>
</tr>
<tr>
<td>β2-AR</td>
<td>sense 5’-ACCCACCAGGAAGCCTCAACTGCT-3’</td>
</tr>
<tr>
<td></td>
<td>antisense 5’-GCCTATCCAATTAGGATGTAAACCTCC-3’</td>
</tr>
<tr>
<td>β3-AR</td>
<td>sense 5’-TGCCCTGAACCTGGCTATGCGG-3’</td>
</tr>
<tr>
<td></td>
<td>antisense 5’-CCCAGTGCAGTGGTGAGGTATGA-3’</td>
</tr>
<tr>
<td>β-Actin</td>
<td>sense 5’-AAAGACCTGTACGCAACACAGTGCTGTCTGG-3’</td>
</tr>
<tr>
<td></td>
<td>antisense 5’-GGTCATACTCTGTCTGGATCCACATCTGC-3’</td>
</tr>
</tbody>
</table>

Table 4. β1-, β2- and β3-AR mRNA levels in normal mucosa and cancer tissue

<table>
<thead>
<tr>
<th></th>
<th>Normal mucosa</th>
<th>Cancer tissue</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>β1-AR</td>
<td>0.49 ± 0.11</td>
<td>0.43 ± 0.16</td>
<td>n.s.</td>
</tr>
<tr>
<td>β2-AR</td>
<td>0.19 ± 0.1</td>
<td>0.18 ± 0.2</td>
<td>n.s.</td>
</tr>
<tr>
<td>β3-AR</td>
<td>1.16 ± 0.14</td>
<td>1.88 ± 0.24</td>
<td>0.036</td>
</tr>
</tbody>
</table>

The values are expressed as the number of β-AR mRNA molecules per number of β-actin mRNA molecules (mean value ± SE). p values were calculated using the paired t test. n.s. = Not significant.

References

Upregulation of $\beta_3$-Adrenergic Receptor in Colon Cancer