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Immunohistochemical detection of dopamine D2 receptors in neuroendocrine tumours

Immunohistochemiczna detekcja receptorów dopaminowych D2 w guzach neuroendokrynnych

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Abstract

Background: Recently, dopamine D2 receptors (RD2) have been found to be expressed in neuroendocrine tumours (NET), the tumours which arise from the diffuse neuroendocrine cells. Moreover, successful trials of the treatment of NET with cabergoline — D2 agonist, have been reported. These findings increase the interest of investigating RD2 expression in NET.
Material and methods: The expression of RD2 was investigated immunohistochemically using the antibody which recognises both short (S) and long (L) isoforms of the receptor in 17 NET samples taken from 15 patients.
Results: In 17 NET samples, a positive reaction with the anti-RD2 antibody occurred in 11 cases. In six cases, the localisation of the immunostaining was cytoplasmic and in nine cases it was nuclear. Only in one case was the receptor cell membrane-located, and in two cases the immunoreaction was also localised in the blood vessels walls. The relation between RD2 expression and the grade of malignancy examined by means of Ki-67 antigen expression needs further study. However, preliminary observations indicate that the nuclear localisation of RD2 is linked to higher tumour malignancy. The next investigated question was the co-expression of somatostatin and dopamine receptors. This question seems important because of the perspectives of somatostatin-dopamine chimeras application in NET treatment. In the samples examined by us, RD2 were co-expressed in 5/10 cases with sstr1, in 3/10 with sstr2A, in 2/9 with sstr2B, in 3/10 with sstr3, and in 5/10 with sstr5.
Conclusion: Dopamine D2 receptors are revealed by means of immunohistochemistry in the majority of NET. They exhibit cytoplasmic and/or nuclear localisations, the latter being possibly linked to a higher grade of malignancy, and are often co-expressed with somatostatin receptors (mostly with subtypes1 and 5). **(Pol J Endocrinol 2011; 62 (5): 388–391)**

Key words: *neuroendocrine tumours, dopamine receptors, somatostatin receptors, immunohistochemistry*

Streszczenie

Wstęp: Ostatnio wykryto występowanie receptorów dopaminowych D2 (RD2) w guzach neuroendokrynnych (NET) i doniesiono o skutecznych próbach leczenia NET kabergoliną — agonistą tych receptorów. Zwiększa to znaczenie badań nad ekspresją RD2 w NET.
Materiał i metody: Zbadano 17 wycinków guzów NET pobranych od 15 pacjentów. Badania immunohistochemiczne przeprowadzono z użyciem przeciwciała rozpoznającego zarówno krótką (S), jak i długą (L) izoformę receptora D2.
Wyniki: Pozytywny odczyn z przeciwciałem anty-RD2 występował w 11 spośród 17 badanych przypadków NET. W 6 na 17 badanych wycinków był to odczyn o lokalizacji cytoplazmatycznej, w 9 na 17 odczyn jądrowy, a tylko w jednym przypadku odczyn o lokalizacji błonowej. Ponadto w 2 przypadkach obserwowano odczyn w ścianach naczyń krwionośnych. Podjęto próbę oceny zależności między stopniem złośliwości guza, określonym na podstawie badania antygenu Ki-67, a ekspresją RD2. Wstępne obserwacje wskazują, że lokalizacja jądrowa odczynu na RD2 wiąże się z wyższym stopniem złośliwości nowotworu. Zależność ta wymaga dalszych badań na większym materiale. Ponadto zbadano współwystępowanie receptorów somatostatynowych i dopaminowych. Zagadnienie to ma istotne znaczenie ze względu na perspektywy stosowania chimer somatostatynowo-dopaminowych w leczeniu NET. W badanych przez autorów pracy guzach cytoplazmatyczne receptory D2 występowały w 5 spośród 10 przypadków, łącznie z sstr1, w 3/10 z sstr2A, w 2/9 z sstr 2B, 3/10 z sstr3 i 5/10 z sstr5.
Wnioski: Receptory dopaminowe D2 wykrywa się za pomocą metody immunohistochemicznej w większości NET. Mają one lokalizację cytoplazmatyczną i/lub jądrową. Lokalizacja jądrowa wydaje się wiązać z większym stopniem złośliwości nowotworu. RD2 wykazują często koekspresję z receptorami somatostatynowymi, zwłaszcza podtypami 1 i 5. **(Endokrynol Pol 2011; 62 (5): 388–391)**

Słowa kluczowe: *guzy neuroendokrynne, receptory dopaminowe, receptory somatostatynowe, immunohistochemia*

Introduction

It is well known that dopamine, acting via the D2 subtype of dopamine receptors (RD2) exerts anti-secretory, antiproliferative and pro-apoptotic effects on prolactin cells of the normal anterior pituitary gland and prolactin-secreting pituitary adenomas [1–4]. Nowadays, dopamine agonists comprise the first line of treatment for prolactinomas [5]. Recently, a high incidence of dopamine D2 receptors (RD2) has

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been found in neuroendocrine tumours (NET) [6, 7] and in neuroendocrine tumoural cell lines *in vitro* [8]. Moreover, promising trials of the treatment of NET with cabergoline — D2 agonist have been reported [6, 9]. These findings increase the interest of investigating RD2 expression in NET. Moreover, it is well known that NET express somatostatin receptors which are targets of somatostatin analogue therapy. It is also known that somatostatin and dopamine receptors can heterodimerise and this heterodimerisation may result in enhanced activity [10]. Thus, co-expression of dopamine and somatostatin receptors in NET cells may provide a new therapeutic option in the treatment of these tumours with dopamine-somatostatin chimeras [11, 12]

Material and method

Patients and samples

We studied 17 samples of neuroendocrine tumours in 15 patients. The samples included six intestinal "carcinoids", five bronchial "carcinoids", four hepatic metastases of unknown origin, one bone metastasis of unknown origin, one thymic neuroendocrine tumour and one pancreatic neuroendocrine cancer. Three patients presented ectopic ACTH-dependent Cushing syndrome. The study was approved by the Local Bioethical Committee, decision number RNN/191/09/ /KE dated 22 September, 2009.

Immunohistochemistry

RD2 immunostaining was performed using the rabbit anti-human dopamine receptor D2 polyclonal antibody. This antibody was raised against the 28 amino acid sequence from the human D2 receptor within the cytoplasmic loop 3 and recognises both the long and short form of the human receptor. The antibody was purchased from Chemicon International (Temecula, CA, USA). It was applied in working dilution 1:100. Ki-67 antigen-immunopositive cell nuclei were stained using the MIB-1 antibody (Dako-Cytomation) and estimated in 500 randomly counted cell nuclei and expressed as a percentage. For detection of the particular subtypes of somatostatin receptors (rsst1-5), we used the primary antibodies raised against the specific regions of sst receptor proteins, obtained from Gramsch Laboratories (Schwabhausen, Germany). The technical details of somatostatin receptors immunohistochemistry have been previously published elsewhere [13, 14] Visualisation of reactions was done by means of the streptavidinbiotin-peroxidase technique with use of StreptABC/HRP kit (Dako-Cytomation) and 3,3′-diaminobenzidine as chromogen. The sections proceeded without the primary antibody served as controls.

Results

A positive reaction with the anti-D2 antibody was observed in 11 NET samples. In 6/17 cases, the immunostaining was cytoplasmic (Figure 1), and in nine cases it was nuclear (Figure 2). This nuclear staining usually was accompanied by cytoplasmic immunopositivity, but sometimes appeared in spite of the immunonegative cytoplasm (Figure 3). Only in one case was the receptor immunostaining localised in cell membranes, and in two samples also in the blood vessels walls (Figures 2, 4). The relation between RD2 expression and the grade of malignancy was examined by means of Ki-67 expression in cell nuclei. It was shown that the cytoplasmic localisation of D2 receptor immunostaining was more frequent (5/10 cases) in tumours presenting lower Ki-67 indices (< 2%) compared to those with higher Ki-67 values (Ki-67 equal to or higher than 2%, 3/10 cases). In contrast, in NET samples presenting the Ki-67 index equal to or higher than 2%, the nuclear localisation was prevalent (Ki-67 equal or > 2%, 6/7 samples, Ki-67 < 2%, 2/7 samples).

The next investigated question was the co-expression of somatostatin and dopamine receptors. In the samples examined by us, RD2 were coexpressed in 5/10 cases with sstr1, in 3/10 with sstr2A, in 2/9 with sstr2B, in 3/10 with sstr3 and in 5/10 with sstr5.

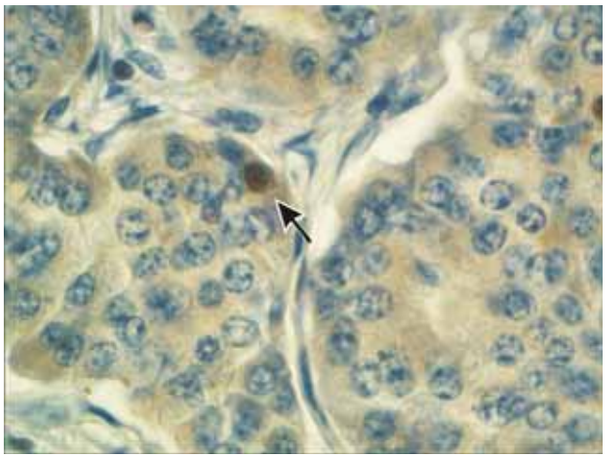

Figure 1. *Immunostaining with anti-RD2 antibody of the intestinal NET ("carcinoid") in a 32 year-old woman. Moderate positive immunostaining in cytoplasm of tumoural cells and strong positive reaction in one cell nucleus. Original magnification × 400*

Rycina 1. *Odczyn immunohistochemiczny z przeciwciałem anty-RD2 w jelitowym NET („rakowiaku") u 32-letniej kobiety. Umiarkowanie dodatni odczyn w cytoplazmie komórek guzowych i silnie dodatni w jednym jądrze komórkowym. Powiększenie oryginału × 400*

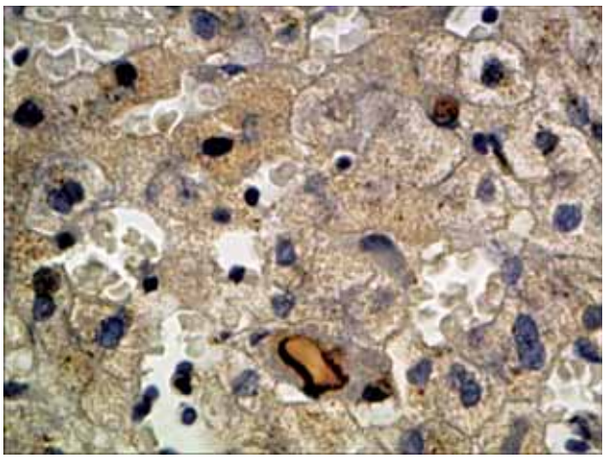

Figure 2. *Immunostaining with anti-RD2 antibody of the thick needle biopsy of the hepatic metastasis of intestinal (?) NET ("carcinoid") in a 68 year-old man. Weak positive immunostaining in cytoplasm of tumoural cells, strong positive reaction in some cell nuclei and in the wall of intratumoural blood vessel. Original magnification × 400*

Rycina 2. *Odczyn immunohistochemiczny z przeciwciałem anty-RD2 w biopsji gruboigłowej przerzutu do wątroby jelitowego (?) NET („rakowiaka") u 68-letniego mężczyzny. Słabo dodatni odczyn w cytoplazmie komórek guzowych, silnie dodatni odczyn w niektórych jądrach komórkowych oraz w ścianie wewnątrzguzowego naczynia krwionośnego. Powiększenie oryginału × 400*

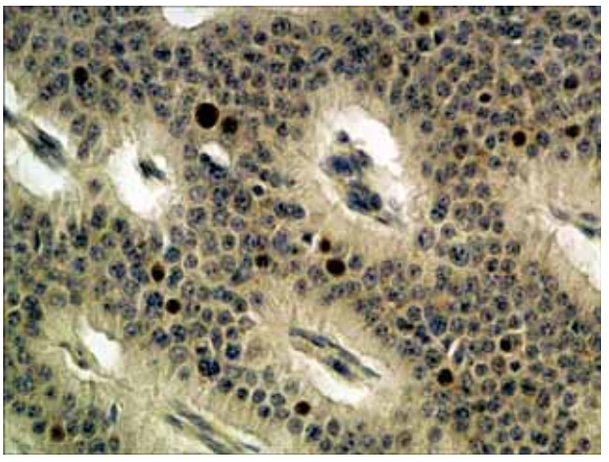

Figure 3. *Intestinal NET ("carcinoid") in a 34 year-old woman. Strong positive immunostaining with anti-RD2 antibody of numerous cell nuclei in spite of negative staining in cytoplasm of tumoural cells. Original magnification × 200*

Rycina 3. *Jelitowy NET („rakowiak") u 34-letniej kobiety. Silnie dodatni odczyn immunohistochemiczny z przeciwciałem anty-RD2 w licznych jądrach komórkowych mimo negatywnego wybarwienia cytoplazmy komórek guzowych. Powiększenie oryginału × 200*

Discussion

Our findings confirm the earlier data (cited in the Introduction) that a majority of NET expresses the D2 receptors (approx. 65% of investigated tumours). Although dopamine receptors belong to the family of G-protein-linked membrane receptors, membrane-located immunostaining with the RD2-antibody was exceptional (only one case in our material). The prevalence of cytoplasmic localisation of immunostaining has also been observed in the case of other "membrane" receptors such as somatostatin and angiotensin receptors [14, 15]. This localisation of the immunoreactive receptor proteins may be explained in two ways. Firstly, it may be a result of receptor internalisation as an effect of the agonist stimulation. Secondly, it may represent *de novo* synthesised receptor protein within the endoplasmic reticulum. In the case of the somatostatin receptors SSTR2 and SSTR5, the membrane-linked localisation is not a condition of the biological functionality [14]. Because our patients were not treated with dopamine agonists, either before or after the sample collection, we cannot answer the question whether the observed D2 receptors were functional. Unexpectedly, we found that the positive immunostaining may concern also the subpopulation of cell nuclei. Such a nuclear localisation

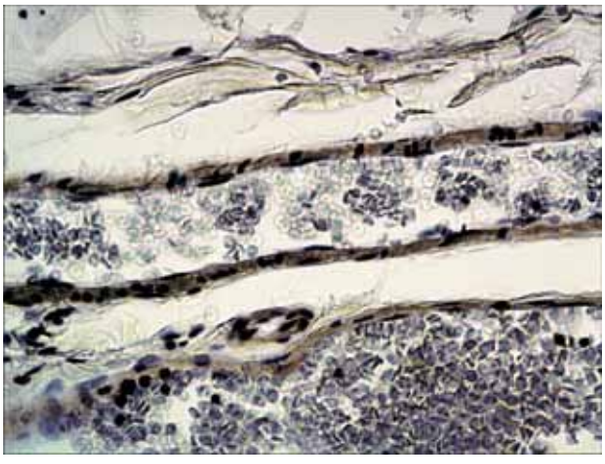

Figure 4. *Positive immunostaining with anti-RD2 antibody in walls of peritumoural blood vessels. Same patient as in Figure 1. Original magnification × 400*

Rycina 4. *Dodatni odczyn immunohistochemiczny z przeciwciałem anty-RD2 w ścianach okołoguzowych naczyń krwionośnych. Ta sama pacjentka co na rycinie 1. Powiększenie oryginału × 400*

has not yet been described in NET, but was observed by us and by other authors applying the immunohistochemical method to detect the RD2 in pituitary adenomas [16–18]. The meaning of such nuclear localisation remains unclear and should be investigated in further studies involving larger materials. However, the preliminary study suggests that the nuclear localisation is linked to higher tumour malignancy. Only in two

cases did we observe RD2 immunopositivity within the intratumoural blood vessels walls, although such localisation is common in pituitary adenomas [16]. This unusual localisation may reflect the involvement of dopamine in the angiogenesis control, as suggested by Chakroborty et al. [19].

The next investigated question was the co-expression of somatostatin and dopamine receptors. This question seems important because of the perspectives of application of somatostatin dopamine chimeras in NET treatment [11, 12, 20]. Our data indicates that the D2 receptors are mostly co-expressed with somatostatin receptors of subtypes SSTR 1 and 5. This is not surprising, because our earlier study, based on the same material, revealed that SSTR1 and 5 are the dominant forms of somatostatin receptors in NET [21].

Summing up, dopamine D2 receptors are revealed by means of immunohistochemistry in the majority of NET. They exhibit the cytoplasmic and/or nuclear localisation (the latter possibly linked to a higher grade of malignancy) and RD2 are often co-expressed with somatostatin receptors (mostly with subtypes 1 and 5).

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