Anti-Cancer Drugs 2001, **12,** pp. 133–136

Report

Neuropeptide receptor status in human tumor cell lines

Thierry Petit,[1] Karen K Davidson,[1] Richard A Lawrence,[1] Daniel D von Hoff[1] and Elzbieta Izbicka[1]

[1]Institute for Drug Development, Cancer Therapy Research Center, San Antonio, TX 78245, USA.

Tumor types expressing a neuroendocrine phenotype secrete neuropeptides with paracrine or autocrine growth factor activity. The efficacy of these paracrine or autocrine loops depends on the expression of specific receptors on tumor cells. Once specific receptors are identified, specific neuropeptide antagonists disrupting paracrine and autocrine loops could be potential treatments in neuropeptide-secreting tumors. In the present study, 11 human tumor cell lines representing astrocytoma, lymphoma, and pancreatic, prostate, lung and colon carcinomas were examined for expression of five different neuropeptide receptors (cholecystokinin, neurotensin, vasopressin, tachykinine substance P and cannabinoid) using RT-PCR and radioligand binding. The presence of various neuropeptide receptors in different human cancer cell lines supports development of new antitumor treatments based on disruption of neuropeptide autocrine growth pathways. [© 2001 Lippincott Williams & Wilkins.]

Key words: Cannabinoid, cholecystokinin, neuropeptide receptor, neurotensin, tachykinine substance P, vasopressin.

Introduction

Tumor types expressing a neuroendocrine phenotype secrete neuropeptides with paracrine or autocrine growth factor activity.[1,2] The efficacy of these paracrine or autocrine loops depends on the expression of specific receptors on tumor cells. Once specific receptors are identified, specific neuropeptide antagonists disrupting paracrine and autocrine loops could be potential treatments in neuropeptide-secreting tumors.[3,4]

In the present study, 11 human tumor cell lines were examined for five different neuropeptide receptor expression using reverse transcriptase polymerase chain reaction (RT-PCR) and radioligand binding. These results could determine which tumor types might be appropriate targets for neuropeptide antagonists.

Study supported by a grant from Sanofi Winthrop Inc.

Correspondence to E Izbicka, CTRC Institute for Drug Development, 14960 Omicron Drive, San Antonio, TX 78245, USA.
Tel: (+1) 210 677-3879; Fax: (+1) 210 677-3886;
E-mail: eizbicka@saci.org

Materials and methods

Neuropeptides

Five neuropeptides were studied: cholecystokinin (CCK),[5] neurotensin (NT),[1] vasopressin (VP),[1] the tachykinine substance P (NK)[5] and the cannabinoids family (CB).[1] Because each neuropeptide interacts with different receptor subtypes according to the organ, the present study focused on the following receptors: the two forms of the cholecystokinin receptors, CCK_A mainly found in the gut and CCK_B distributed mainly in the brain; one subtype of the neurotensin receptors, NT_1 mainly distributed in the brain; the subtype of vasopressin receptors essentially present in peripheral tissues, VP_{1a}; the subtype of tachykinin receptors with the highest affinity for substance P, NK_1; and the subtype of cannabinoid receptors mainly distributed in the central nervous system, CB_1.

Tumor cell lines

Eleven human tumor cell lines were studied: three pancreatic carcinoma cell lines (MiaPaCa-2, RPW-2 and PANC-1), two prostate adenocarcinoma cell lines (LNCaP and DU-145), three small cell lung cancer cell lines (UMC-5, NCI-H345 and NCI-H69), one colon carcinoma cell line (HT-29), one astrocytoma cell line (U373), and one lymphoma cell line (Raji). These cell lines were chosen because occasional reports had described their growth stimulation by neuropeptides.

The UMC-5 cells were a gift from Dr Gary Gamble (SUNY, Syracuse, NY). The RPW-2 cells were a gift from Cancer Therapy and Research Center, *In Vivo* Laboratory (San Antonio, TX) and all other cell lines were purchased from ATCC (Rockville, MD). The cells were grown in appropriate culture medium containing 10% heat-inactivated fetal bovine serum (HI-FBS) at 37°C in a humidified incubator with 5% CO_2. After several passages to allow full adaptation, the cells were seeded at a high density in four T-150 flasks. The cells were then grown in the regular medium supplemented with 10% HI-FBS for 3–5 days depending on the rate of proliferation.

RNA isolation and RT-PCR

Two flasks containing more than 2×10^7 cells from each cell line were used to isolate polyadenylated mRNA using Fast Track (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. mRNA isolation yields were in the range of 5–80 μg/g of tissue. Complementary DNA was synthesized from mRNA by murine leukemia virus RT. PCR used human-specific oligonucleotide primers for the six receptors, designed using Oligo software (Table 1). The primers were synthesized at the Center for Advanced DNA Technology at the University of Texas Health Science Center (San Antonio, TX). Although no cell lines express all receptors, the receptors not expected to be present in a given cell line were treated as negative controls. Human β-actin was used as a positive control. The following conditions were used for the PCR: 94°C during 2 min, then 94°C during 30 s/60°C during 30 s/72°C during 30 s for 30 cycles. The PCR products were electrophoresed in agarose gels and visualized with ethidium bromide stain. All reactions were run twice.

Ligand binding study

^{125}I-labeled octapeptide CCK8, NT_1, VP_{1a}, NK_1 and ^{3}H-labeled CB_1 agonist CP 55940 were available as radioligands, and were purchased from DuPont New England Nuclear (Boston, MA). Octapeptide CCK8 binds on both CCK_A and CCK_B receptors. For the ligand binding experiment, a transfected cell control was included. This control was generated by transiently transfecting the cDNA expressing the appropriate receptor type into COS-7 cells as previously described.[6] Untransfected cells were also used as negative controls. The control plasmid for the CB_1 receptor was not available.

The specific binding conditions for each ligand were different. The buffer and membrane conditions from recent binding protocols were followed for each ligand: CCK ligand binding,[7] CB_1 ligand binding,[8] NT_1 ligand binding,[9] VP_{1a} ligand binding[10] and NK_1 ligand binding.[11]

Results

Pancreatic cancer cell lines

Three different neuropeptide receptors were detected in the three pancreatic cancer cell lines (Table 2) with both ligand binding and RT-PCR. CCK_A was detected in MiaPaCa-2, RWP-2 and PANC1 cell lines, and CCK_B was only detected in the MiaPaCa-2 cell line. CB_1 was detected in MiaPaCa-2 and PANC1.

Table 1. Oligonucleotide primers for neuropeptide receptors cDNA amplification

	Primers	Product size (bp)
CCK_A	5′-AGG ACA GCG ATG GGT GTT AC-3′ (791–810) 5′-AGA ACC TGG ACA GAG AGG CTC-3′ (1249–1229)	459
CCK_B	5′-GCA GTG ATC TTC CTG ATG AGC-3′ (184–204) 5′-AAG AGC AGA AGC AGC AGT ACG-3′ (677–657)	494
NT_1	5′-CGA CAT CTA CTC CAA AGT GCT G-3′ (174–195) 5′-CCA CCA TGG GGA ATA TGA AG-3′ (751–732)	404
CB_1	5′-GTC TGA GGA TGG GAA GGT ACA G-3′ (963–984) 5′-TCT TGA CCG TGC TCT TGA TG-3′ (1366–1347)	578
NK_1	5′-ACC CCA TCA GTT CGT GCA AC-3′ (78–97) 5′-TCC CAC TAT GGT GTA TGC ATA GC-3′ (675–653)	572
V_{1a}	5′-CAA ATG TGC TGG GAC ATC AC-3′ (322–341) 5′-GTC ACG ATC ACA AAA GTC ATC TTC-3′ (893–870)	271
β-actin	5′-TGA CTA CCT CAT GAA GAT CCT CA-3′ (558–580) 5′-TTC GTG GAT GCC ACA GGA C-3′ (828–810)	271

Table 2. Neuropeptide receptor status in human tumor cell lines (ligand binding method/RT-PCR)

		CCK_A	CCK_B	NT_1	CB_1	NK_1	V_{1a}
MiaPaCa-2	pancreas	+/+	+/+	−/+	+/+	−/−	+/−
RWP-2	pancreas	+/+	−/−	−/−	−/+	−/−	−/−
PANC1	pancreas	+/+	−/−	−/−	+/+	−/−	−/−
LNCaP	prostate	+/+	−/−	−/−	−/−	−/−	−/−
DU-145	prostate	−/−	−/−	−/−	−/−	−/−	−/−
NCI-H69	lung	+/+	+/+	+/−	−/−	−/−	−/−
UMC-5	lung	−/−	−/−	−/−	−/−	−/−	−/−
NCI-H345	lung	−/−	−/−	−/−	−/−	+/−	−/−
HT-29	colon	−/−	−/−	−/−	−/−	−/−	−/−
U373	astrocytoma	+/−	−/−	−/−	+/+	+/−	−/−
Raji	lymphoma	−/−	−/−	+/−	+/−	−/−	−/−

Lung cancer cell lines

The UMC-5 and NCI-H345 cell lines did not express any neuropeptide receptor. In contrast, CCK_A and CCK_B were detected in the NCI-H69 cell line with ligand binding study and RT (Table 2).

Prostate cancer cell lines

The DU-145 cell line did not express any neuropeptide receptor, while the LNCaP cell line expressed CCK_A receptor (Table 2).

Other cancer cell lines

No neuropeptide receptor was detected in the HT-29 colon cancer cell line or the Raji lymphoma cell line with either method. CB_1 receptors were detected in the U373 astrocytoma cell line (Table 2).

Discussion

In the present report, neuropeptide receptors have been documented in a panel of human tumor cell lines using both a ligand binding study and RT-PCR. This supports a rational basis to study neuropeptide antagonists in human cancer. Previous reports have already studied neuropeptide receptor expression in cancer cell lines, with a few functional studies demonstrating that neuropeptides could stimulate growth of cancer cell lines.[12,13] CCK receptor expression has already been reported in pancreatic tumor cell lines[14] and small cell lung cancer cell lines.[15] The present study is the first one demonstrating CCK_A expression in the androgen receptor-positive prostate cancer LNCaP cell line. Receptors of neurotensin were shown in small cell lung cancer, prostate cancer, colon cancer and pancreatic cancer cell lines in previous studies,[16–18] but were not detected by both the ligand binding and the RT-PCR as they were in the present study. Previous studies on CB_1 in human cell lines were limited to astrocytoma[19] and B lymphocytes.[20] The expression of CB_1 receptor in the pancreatic cancer MiaPaCa-2, RWP-2 and PANC1 cell lines and the NCI-H446 small cell lung cancer cell line in the present study was also demonstrated. Functional receptors for substance P have already been reported in astrocytoma cell lines,[21] and for substance P and vasopressin in small-cell lung cancer lines.[22,23] These results were not confirmed with the ligand binding method and the RT-PCR in the present study.

Conclusions

The presence of various neuropeptide receptors in different human cancer cell lines supports the development of new antitumor treatments based on the disruption of neuropeptide autocrine growth pathways.

Acknowledgments

We are grateful to Dr Robert Klebe (University of Texas Health Science Center, San Antonio, TX) for help with the PCR experiments and for numerous productive discussions.

References

1. Woll P. Neuropeptide growth factors and cancer. *Br J Cancer* 1991; **63**: 469–75.
2. Treston AJ, Mulshine CF. Control of tumor cell biology through regulation of peptide hormone processing. *J Natl Cancer Inst Monogr* 1992; **13**: 169–75.

3. Mahmoud S, Staley J, Taylor J, *et al*. [Psi^{13,14}] bombesin analogues inhibit growth of small cell lung cancer *in vitro* and *in vivo*. *Cancer Res* 1991; **51**: 1798–802.
4. Cuttitta F, Carney D, Mulshine J, *et al*. Bombesin-like peptides can function as autocrine growth factors in human small-cell lung cancer. *Nature* 1985; **316**: 823–6.
5. Betancur C, Azzi M, Rostene W. Nonpeptide antagonists of neuropeptide receptors: tools for research and therapy. *Trends Pharmacol Sci* 1997; **18**: 372–83.
6. Kolakowski L, Lu B, Gerard C, Gerard N. Probing the 'message: address' sites for chemosttractant binding to the C5a receptor: mutagenesis of hydrophilic and proline residues within the transmembrane segments. *J Biol Chem* 1995; **270**: 18077–82.
7. Schmitz F, Pratt D, Wu M, Kolakowski L, Beinborn M, Kopin A. Identification of cholecystokinin-B/gastrin receptor domains that confer high gastrin affinity: utilization of a novel *Xenopus laevis* cholecystokinin receptor. *Mol Pharmacol* 1996; **50**: 436–41.
8. Felder C, Joyce K, Briley E, *et al*. Comparison of the pharmacology and signal transduction of the human cannabinoid CB_1 and CB_2 receptors. *Mol Pharmacol* 1995; **48**: 443–50.
9. Cusack B, Groshan K, McCormick D, *et al*. Chimeric rat/human neurotensin receptors localize a region of the receptor sensitive to binding of a novel, species-specific, picomolar affinity peptide. *J Biol Chem* 1996; **271**: 15054–9.
10. Mouillac B, Chini B, Balestre M, *et al*. The binding site of neuropeptide vasopressin V_{1a} receptor. Evidence for a major localization within transmembrane regions. *J Biol Chem* 1995; **270**: 25771–7.
11. Jensen C, Gerard N, Schwartz T, Gether U. The species selectivity of chemically distinct tachykinin nenpeptide antagonists is dependent on common divergent residues of the rat and human neurokinin-1 receptors. *Mol Pharmacol* 1994; **45**: 294–9.
12. Watson S, Durrant L, Morris D. Gastrin: growth enhancement effects on human gastric and colonic tumor cells. *Br J Cancer* 1989; **59**: 554–9.
13. Smith JP, Kramer ST, Solomon TE. CCK stimulates growth of six pancreatic cancer cell lines in serum-free medium. *Reg Peptides* 1991; **32**: 341–9.
14. Palmer Smith J, Rickabaugh C, McLaughlin P, Zagon I. Cholecystokinin receptors and PANC-1 human pancreatic cancer cells. *Am J Physiol* 1993; **265**: G149–55.
15. Denyer J, Gray J, Wong M, Stolz M, Tate S. Molecular and pharmacological characterization of the human CCK_B receptor. *Eur J Pharmacol* 1994; **268**: 29–41.
16. Allen A, Carney D, Moody T. Neurotensin binds with high affinity to small cell lung cancer cells. *Peptides* 1988; **9**(suppl 1): 57–61.
17. Sehgal I, Powers S, Huntley B, Powis G, Pittelkow M, Maihle N. Neurotensin is an autocrine trophic factor stimulated by androgen withdrawal in human prostate cancer. *Proc Natl Acad Sci USA* 1994; **91**: 4673–7.
18. Yamada M, Ohata H, Momose K, Yamada M, Richelson E. Pharmacological characterization of SR 48692 sensitive neurotensin receptor in human pancreatic cancer cells, MIA Paca-2. *Res Commun Mol Pathol Pharmacol* 1995; **90**: 37–46.
19. Boulaboula M, Bourrie B, Rinaldi-Carmiona M, Shire D, Le Fur G, Casellas P. Stimulation of cannabinoid receptor CB_1 induces *krox-24* expression in human astrocytoma cells. *J Biol Chem* 1995; **270**: 13973–80.
20. Derocq J, Segui M, Marchand J, Le Fur G, Casellas P. Cannabinoids enhance human B-cell growth at low nanomolar concentrations. *FEBS Lett* 1995; **369**: 177–82.
21. Johnson C. Characterization of receptors for substance P in human astrocytoma cells: radioligand binding and inositol phosphate formation. *J Neurochem* 1992; **22**: 471–6.
22. Woll P, Rozengurt E. A neuropeptide antagonist that inhibits the growth of small cell lung cancer *in vitro*. *Cancer Res* 1990; **50**: 3968–73.
23. Hong M, Moody T. Vasopressin elevates cytosolic calcium in small cell lung cancer cells. *Peptides* 1991; **12**: 1315–9.

(Received 24 October 2000; accepted 10 November 2000)