Available online at www.sciencedirect.com
SCIENCE DIRECT®
Cancer Letters 222 (2005) 95–105
CANCER Letters
www.elsevier.com/locate/canlet

Analysis of the cellular and molecular mechanisms of trophic action of a misspliced form of the type B cholecystokinin receptor present in colon and pancreatic cancer☆

Zhi-Jie Cheng, Kaleeckal G. Harikumar, Wei-Qun Ding, Eileen L. Holicky, Laurence J. Miller*

Mayo Clinic Cancer Center, Department of Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic in Scottsdale, 13400 East Shea Boulevard, Scottsdale, AZ 85259, USA

Received 20 May 2004; received in revised form 5 August 2004; accepted 2 September 2004

Abstract

Gastrin and cholecystokinin (CCK) have trophic action on cells expressing wild type A or B CCK receptors. Potential relevance to pancreatic and colonic cancers was raised by the demonstration of a misspliced type B CCK receptor that, when expressed in Balb3T3 cells, had constitutive activity to stimulate intracellular calcium. We attempted to confirm and extend this observation in CHO cells by establishing lines expressing similar densities of variant or wild type B CCK receptor. While both were capable of normal binding and agonist-induced signaling, neither expressed constitutive signaling and both had similar basal growth. Agonist stimulation of cells expressing misspliced receptor had greater increases in calcium and greater growth rates than control cells despite similar MAP kinase phosphorylation responses. Thus, this variant receptor can potentate peptide-stimulated signaling and trophic action and may contribute to the proliferation of neoplasms expressing it.

Keywords: G protein-coupled receptor; Signaling; Peptide hormone; Cholecystokinin; Gastrin

1. Introduction

Under normal conditions, the gastrointestinal peptides gastrin and cholecystokinin (CCK) act as growth factors for the gastric mucosa and the pancreas, respectively [1,2]. These actions likely are important for tissue maintenance and preservation. Of interest, the growth-promoting effects of these peptides have also been reported in a variety of human cancers and cell lines derived from them, including pancreatic, gastric, colonic and small cell lung tumors

Abbreviations: CCK, cholecystokinin; CCKBR, type B CCK/gastrin receptor; CCKBR-i4 variant, the misspliced form of the type B CCK receptor that retains intron 4; CHO, Chinese hamster ovary; MAP kinase, mitogen-activated protein kinase; KRH, Krebs–Ringers–HEPES medium.

☆ This work was supported by grants from the National Institutes of Health, DK32878, the Fiterman Foundation, and by the Kendall-Mayo Fellowship Award (Z.-J.C.).

* Corresponding author. Tel.: +1 480 301 6650.
E-mail address: miller@mayo.edu (L.J. Miller).

0304-3835/$ - see front matter © 2004 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.canlet.2004.09.008

[2–4]. It is not yet clear whether these growth effects reflect similar normal processes or whether these might reflect pathological growth stimulation that is not responsive to normal feedback inhibition or that is quantitatively excessive. Such a trophic influence could even contribute to the development, growth, or progression of these neoplasms.

Signaling events normally initiated by CCK include inositol phospholipid hydrolysis, intracellular calcium influx, protein kinase C activation, and MAP kinase activation. Among these, intracellular calcium influx and MAP kinase phosphorylation have attracted the most attention as molecular mechanisms associated with growth-promoting effects [4]. Gastrin has been reported to stimulate AR42J cell growth through induction of p42/44 MAP kinase [5]. Its growth-promoting effect on GH3 cells, where it fails to activate MAP kinase, is through a calcium-dependent mechanism [6]. This is also true in receptor-bearing CHO cells, where gastrin stimulates DNA synthesis in a calcium-dependent manner [7]. Logsdon et al. reported that short-term CCK treatment induces similar calcium responses, but has different growth effects in CHO and Swiss 3T3 cells engineered to express type B CCK receptors [8]. Thus, the growth-promoting effects of these peptides seem to be not only dependent on complement of receptors and their density, but also on the cellular environment utilized.

While there are scattered reports of the presence of wild type CCK receptors (type A and type B) on colonic and pancreatic cancers [3,9–12], this has not been common, prominent, or reproducible. In contrast, there have been recent reports to suggest the presence of a novel receptor variant of the type B CCK receptor (CCKBR-i4) in a high proportion of colonic [13] and pancreatic [14,15] carcinomas. Due to intron 4 retention from RNA missplicing, this receptor variant contains 69 additional amino acid residues in its third intracellular loop region. Balb3T3 cells expressing this receptor variant were shown to exhibit constitutive, oscillatory increases in intracellular calcium, and a ligand-independent increase in growth rate compared with cells expressing wild type B CCK receptor [13]. While these observations provide important insight into a potential role for this receptor variant in stimulating human cancer cell proliferation, it is possible that the unique cellular environment contributed to the response observed in that study.

Here, we characterized the same abnormal spliceoform of the type B CCK receptor expressed in Chinese hamster ovary cell lines, comparing these with analogous lines expressing similar density of wild type CCK receptors. We studied these cells for constitutive activity and peptide-stimulated signaling and proliferation, using both gastrin and CCK. Of note, we observed substantial differences from that previously reported [13], with no constitutive calcium response and no ligand-independent growth in the CHO cells expressing the CCKBR-i4 variant. Despite its normal expression and binding properties, the CCKBR-i4 variant displayed a significant increase in peptide-induced intracellular calcium influx compared with wide type receptor. However, peptide-stimulated p42/44 MAP kinase phosphorylation was similar in the two cell lines. Activation of CCKBR-i4 variant by peptides demonstrated modest but significant promotion in the rate of cell growth. Thus, our results continue to focus interest on this missplicing event as a potential contributor to the abnormal growth and progression of these neoplasms, and suggest that this type B CCK receptor variant may contribute to the proliferative effects of peptides through mechanisms that are partially independent of MAP kinase activation.

2. Experimental methods

Preparation of type B CCK receptor constructs. A type B CCK receptor minigene construct containing the fourth intron was previously established from Panc-1 cells in our laboratory [14]. The fourth intron in this minigene was PCR amplified and inserted into a wild type human type B CCK receptor construct (CCKBR) [16] by creating an *NheI* site, which was later removed. The single base changes ($A^{813} \rightarrow T$, $A^{828} \rightarrow T$, and $C^{1017} \rightarrow A$, not affecting coding) were made at the $5'$ and $3'$ splice boundaries of intron 4 to ensure the retention of the fourth intron in the mature mRNA. The human CCKBR and the variant receptor (CCKBR-i4) constructs were tagged with influenza hemagglutinin (HA) epitope tags at each amino-terminus. The QuickChange™ Site-Directed Mutagenesis Kit was used for these changes.