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Research Report

Promoter analysis of human glutamate carboxypeptidase II

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ABSTRACT

The expression of glutamate carboxypeptidase II (GCP II) is reduced in selective brain regions in schizophrenic patients. To investigate transcriptional mechanisms regulating the human GCP II gene, a 3460 bp DNA fragment comprised of the proximal 3228 bp of 5′ untranscribed sequence and first 232 bp of 5′ UTR portion of this gene was cloned into the mammalian luciferase reporter gene vector pGL3-Basic. Transfection assays in human astrocyte-derived SVG and human prostate tumor-derived LNCaP cells demonstrated that constructs with 3460, 1590 and 761 bp portions of 5′ region of human GCP II gene were able to drive the luciferase reporter gene. Additional deletion constructs showed that in the SVG cell line, constructs with 511 and 411 bp of GCP II gene fragments yielded highest transcriptional activity, with declining activity upon further removal of 5′ sequences. 15 bp of the promoter 5′ to a 225 bp GCP II fragment were essential for luciferase expression. Thus, in the SVG cells, the proximal 240 bp of the human GCP II promoter (232 bp of the 5′ UTR and 8 bp of 5′ untranscribed sequences) may represent the core promoter. Further, while a LyF-1 site lies within and overlaps a transcription start site in the 15 bp sequence, site-directed mutagenesis shows that LyF-1 is not the transcription initiator for the "TATA and CAAT" box lacking GCP II gene in the SVG cells. Finally, pattern differences in GCP II gene promoter expression in SVG and LNCaP cells suggest that sequences beyond 240 bp may be important for tissue-specific GCP II expression.

1. Introduction

Glutamate carboxypeptidase II (GCP II), also known as NAALADase (*N*-acetylated-α-linked acidic dipeptidase), was first identified as the enzyme that hydrolyzes the neuropeptide *N*-acetyl-aspartyl glutamate (NAAG) to *N*-acetyl aspartate (NAA) and glutamate (Blakely et al., 1988; Robinson et al., 1986). NAAG is a highly abundant peptide neurotransmitter in the mammalian central nervous system. It is an antagonist of the NMDA receptor (Sekiguchi et al., 1989; Grunze et al., 1996; Bergeron et al., 2005) and an agonist of the metabotropic glutamate receptor 3, an inhibitor of glutamate release (Wroblewska et al., 1997; Neale et al., 2000). The aggregate effect of NAAG is thus to attenuate NMDA receptor activation. In the brain, GCP II is primarily, if not exclusively, expressed in astrocytes (Slusher et al., 1992; Berger et al., 1999; Ghose et al., 2004; Sacha et al., 2007). Enzymatic and molecular cloning studies further suggest that GCP expressed in the human

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cerebellum, prostate specific membrane antigen (PSMA) expressed on hormone refractory metastatic prostate cancer cells (Israeli et al., 1993), and folate hydrolase I (FOLH1) in the small intestine are identical proteins (Pinto et al., 1996; Luthi-Carter et al., 1998; Halsted et al., 1998; Bacich et al., 2001; Watt et al., 2001). However, their biological functions may significantly differ. Serum levels of PSMA or its DNA increase as prostate cancer progresses; consequently, it is useful as a clinical marker for diagnosis and prognosis of prostate malignancy (Mitsiades et al., 2004; Chen et al., 2005; Papadopoulou et al., 2006). FOLH1 hydrolyzes the polyglutamate side chain from folic acid permitting dietary folate absorption into the small intestine.

Mounting evidence supports the hypothesis that the endophenotype of schizophrenia includes hypofunction of cortico-limbic NMDA receptors (Coyle, 2006). Reduced GCP II expression in brain regions relevant to schizophrenia would reduce NMDA receptor function by interfering with the catabolism of the endogenous NMDA receptor antagonist, NAAG (Tsai et al., 1995); Hakak et al., 2001). Since GCP II regulates both folate absorption and NMDA receptor activation via NAAG hydrolysis, it may also play an important role in the pathophysiology of schizophrenia. Thus, if maternal folate is low and homocysteine levels high, the potential for teratogenic effects may increase the risk for developing schizophrenia (Goff et al., 2004; Picker and Coyle, 2005).

At present, little is known about the regulation of the expression of GCP II. With malignant transformation of prostate cells, GCP II expression markedly increases; for example, 30% of membrane protein is comprised of GCP II/PSMA in the prostate cancer cell line LNCaP (Israeli et al., 1994). Kindled seizures also up-regulate GCP II expression in the rat hippocampus (Meyerhoff et al., 1989). In contrast, reduced expression of GCP II has been reported in selective brain regions in post-mortem studies of patients with schizophrenia (Tsai et al., 1995) while administration of antipsychotic medications and NMDA receptor antagonists increases GCP II expression in rat cerebral cortex (Flores and Coyle, 2003).

GCP II is a class II membrane glycoprotein with an apparent molecular mass of 94 to 100 kDa. O'Keefe et al. (1998), reported that a 1.2 kb portion of the 5′ region of the GCP II gene was able to drive reporter gene expression in prostate but not breast-derived cell lines (O'Keefe et al., 1998). A 2-kb human genomic fragment containing the 5′ untranscribed region of the GCP II gene was identified in prostate and non-prostate cell lines (Good et al., 1999). At the 3′ end of this fragment, 614 bp appears to represent the core promoter required for transcriptional activity. A PSMA enhancer has also been identified within the third intron of FOLH1 (Watt et al., 2001), which apparently confers prostate specific expression of GCP II.

Thus, while GCP II, PSMA and FOLH1 may be identical proteins encoded by the gene now designated as FOLH1, sequences within the untranslated regions of their genes may confer specificity and/or differential tissue specific expression. Our interests focus on the potential role of down-regulation of GCP II in the pathophysiology of schizophrenia. In the present study, we provide the first evidence that GCP II gene promoter-driven transcription occurs in brain astrocytes. Transient transfection assays with nested GCP II promoter deletion-luciferase reporter gene constructs show differential activation of the GCP II promoter in the human astrocyte-derived SVG cell line versus the human prostate cancer cell line LNCaP. In the astrocyte cell line, a core promoter of 240 bp has been identified along with a 15 bp sequence harboring a potential transcription initiation site for the GCP II gene. The latter corresponds to a previously identified transcription initiation site for PSMA/GCP II/FOLH1 and contains a LyF-1 binding motif. While LyF-1 can initiate transcription in genes lacking TATA and CAAT boxes and is expressed at higher levels in the SVG cells, site directed mutational analysis of the GCP II promoter suggests that LyF-1 is not the apparent transcription initiator for the GCP II gene in astrocytes.

2. Results

2.1. *Human library screening and analysis*

By PCR screening of a lambda genomic library with human GCP II specific primers, we identified an ~15 kb genomic clone containing GCP II exon 1 at the 3′ end. Restriction mapping and sequencing confirmed identity to the GCP II gene (O'Keefe et al., 1998, GenBank Accession No. AF007544; Good et al., 1999, GenBank Accession No. AF061571).

2.2. *Expression of GCP II in human cell lines*

To show that GCP II gene expression occurs in the cells to be used in the transient transfection assays with GCP II promoter plasmids, total RNA from SVG, CRL-1718, and LNCaP cells was examined for the presence of GCP II mRNA by RT-PCR. GCP II cDNA amplicons were detected in all three cell lines (Fig. 1A). Highest expression of GCP II mRNA was observed in the LNCaP cells and lowest in the SVG cells. Notably, HeLa cells do not express GCP II as would de predicted by upon regional expression studied (Slusher et al., 1992). Based on densitometric scanning, the relative ratio of GCP II mRNA in the three cell lines, LNCaP:CRL-1718:SVG, was 39:5:1, respectively.

Western blot analysis was also performed on membrane protein from the cells using a polyclonal antibody against the extracellular region of human GCP II. An approximately 100-kDa band was present in the prostate cancer cells LNCaP and an ~120-kDa band in the human brain astrocyte-derived cells SVG and the astrocyte cells CRL-1718 (Fig. 1B). Interestingly, GCP II protein levels showed a similar trend of expression as mRNA with highest and lowest abundance in the LNCaP and SVG cells (LNCaP:CRL-1718:SVG, 42:3:1, respectively).

2.3. *Construction of GCP II promoter deletion constructs*

In order to examine GCP II gene promoter transcriptional functionality, a 3460 bp *Sac*I/*Sma*I fragment from the isolated ~15 kb human GCP II clone was inserted into *Sac*I/*Sma*I restriction sites in the reporter plasmid vector pGL3-Basic to create the plasmid pGL3-hGCP3.4K. This plasmid contains a 3228 bp fragment of 5′-untranscribed region upstream of the transcription start site of the human GCP II gene as previously identified (nt 2062 to nt 5289) and the distal 5′ 232 bp of transcribed but untranslated sequence (nt 5290 to nt 5521) of exon 1 (numbering according to Gene Bank Accession No.