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A novel glutaminase isoform in mammalian tissues

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ABSTRACT

The synthesis of neurotransmitter glutamate in brain is mainly carried out by glutaminase enzymes. This synthesis must be exquisitely regulated because of its harmful potential giving rise to excitotoxic damage. It is noteworthy that two glutaminase isozymes coded by different genes are expressed in the brain of mammals. The need for two genes and two isozymes to support the single process of glutamate synthesis is unexplained, and identifying the role of each glutaminase is an important factor in understanding glutamate-mediated neurotransmission. Multiple transcripts for glutaminase genes and simultaneous expression of glutaminase isoforms have been reported in mammalian tissues and cells. The recent discovery of protein interacting partners widens the possibilities of regulatory mechanisms controlling these biosynthetic enzymes. The expression of distinct isozymes and binding partners may represent the biochemical and molecular basis to achieve fine-tuning control of glutamate synthesis in different cell types or developmental states. In this review, we will briefly summarize recent works on glutaminase proteins in mammals, with particular emphasis on brain studies. We present convergent evidence supporting the existence of a novel glutaminase isozyme in mammalian tissues.

1. Introduction

The enzymes which catalyze cleavage of the side chain amide group in glutamine (Gln) leaving a γ-glutamyl acyl chain and ammonium ions and its subsequent transfer to a specific substrate are called glutamine amidotransferases (EC 2.4.2). In contrast, phosphate-activated glutaminase (EC 3.5.1.2; GA) is a Gln amidohydrolase, a true hydrolytic enzyme because the acyl and ammonium acceptor is water and not other molecule. Krebs in his pioneer work on amino acids metabolism discovered GA while studying the enzymic hydrolysis of Gln in animal tissues (Krebs, 1935). Later on, Errera and Greenstein (1949) first used the term phosphate-activated GA (originally named glutaminase I) to describe the enzymatic activity in kidney, liver, brain and spleen extracts that was greatly increased by added phosphate. In mammals, the enzyme needs inorganic phosphate for *in vitro* activity but it is yet unknown whether phosphate is the true activator *in vivo*.

Some of the main physiological functions of GA include renal ammoniagenesis, nitrogen supply for urea biosynthesis in the liver, synthesis of the excitatory neurotransmitter glutamate (Glu) in the brain, and energy supply for the bioenergetics of many normal and transformed cell types (Kovacevic and McGivan, 1983; Curthoys and Watford, 1995). In brain, although several different precursors have been proposed for the synthesis of transmitter Glu, Gln is considered the most important source through GA reaction (Kvamme, 1984; Nicklas et al., 1987). Besides being the major excitatory neurotransmitter in the CNS (Fonnum, 1984), Glu fulfils many other crucial roles in synaptogenesis, synaptic plasticity, pathogenesis of neuropsychiatric diseases (Conti and Weinberg, 1999 and references therein), synthesis of γ-aminobutyric acid (GABA), and brain energy metabolism (Erecinska and Silver, 1990).

The role of two key genes of Gln metabolism, glutamine synthetase (GS, EC 6.3.1.2) and GA, has also focused considerably attention in tumor biology, because Gln behaves as a central metabolite for growth and proliferation (Matés et al., 2002). The catabolism of Gln has been linked to neoplastic transformation (Kovacevic and McGivan, 1983). The high rate of glutaminolysis observed in a wide variety of tumors would be essential to maintain their proliferative capacity (Souba, 1993). Thus, GA overexpression seems to be a hallmark exhibited by many tumors (Aledo et al., 1994). Studies on experimental and human tumors looking at changes in enzymatic activity and relative mRNA levels of both GS and GA revealed a similar pattern repeated in many cases: a knock-down or repression of GS expression along with an

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Abbreviations: BPTES, bis-2-(5-phenylacetamido-1,2,4-thiadiazol-2-yl)ethyl sulfide; C/EBP, CAAT-enhancer binding protein; CRE, cAMP-responsive element; DON, 6-diazo-5-oxo-norleucine; HNF, hepatocyte nuclear factor; PDZ, PSD95/Dlg/ZO1 domains; PMN, polymorphonuclear neutrophils; RREB, Ras-responsive element binding protein; UTR, untranslated region.

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overexpression of GA (Matsuno and Goto, 1992; Medina et al., 1992; Gebhardt and Williams, 1995).

In conclusion, it can be stated that glutaminase is expressed in most mammalian tissues and cancer cells, but the regulation of its organ- and tumor-specific expression is largely unknown. Therefore, an essential step towards studying the regulation of GA in mammals is the characterization of its tissue-specific isozyme pattern of expression, to which particular attention is given in this short review.

2. Mammalian glutaminase genes and transcripts

In humans, the GA family consists of two main members which are encoded by separate genes in different chromosomes: the *Gls* gene, located in chromosome 2, encodes isoforms known as kidney (K-type) glutaminases, and the *Gls*2 gene, located on chromosome 12, codes for liver (L-type) isozymes (Aledo et al., 2000). Orthologous genes have been described in other mammalian species, like mouse and rat, for *Gls* (Mock et al., 1989) and *Gls*2 (Chung-Bok et al., 1997).

2.1. Gls gene and transcripts

The human *Gls* gene spans 82 kb. By comparison with available human cDNAs, the gene was split into 19 exons (Porter et al., 2002). At least two different transcripts arise from this gene: the KGA mRNA formed by joining exons 1–14 and 16–19, and the alternative spliced transcript named glutaminase C (GAC) mRNA which uses only the first 15 exons, omitting exons 16–19 (Elgadi et al., 1999; Porter et al., 2002) (Fig. 1). The K-type cDNA named

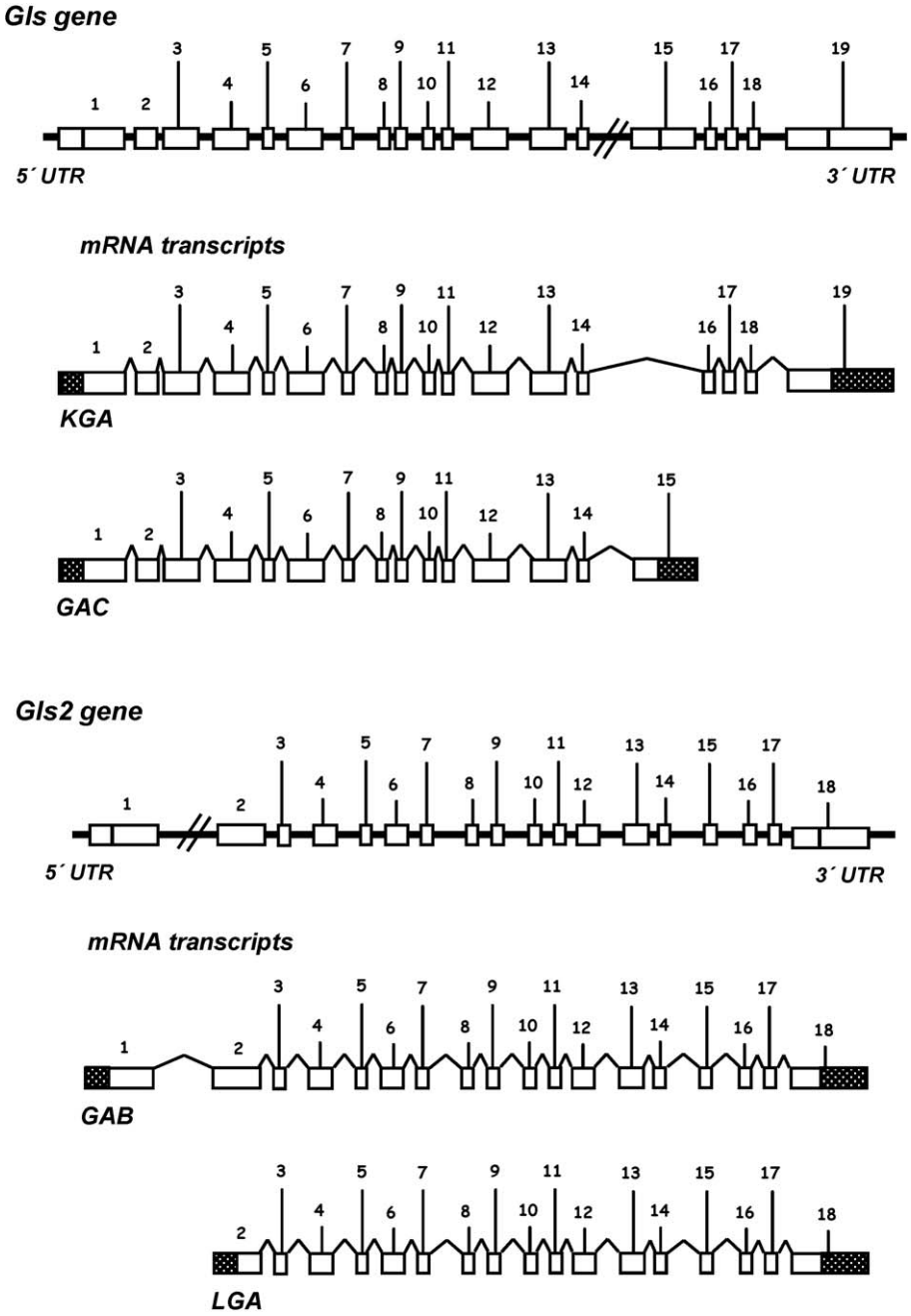

Fig. 1. Schematic diagram showing the mRNA transcripts derived from *Gls* and *Gls*2 mammalian glutaminase genes. Exons are indicated by numbers and depicted as empty boxes. The introns are shown as lines. The 5′- and 3′-UTR regions are separated from their respective exons by a vertical black line in the genes and shown as dotted boxes in the transcripts. The names of identified transcripts are also indicated.