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BRAIN RESEARCH

Research Report

Dicer expression and localization in post-mitotic neurons

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ARTICLE INFO

Article history:
Accepted 31 July 2007
Available online 22 August 2007

Keywords:
MicroRNA
Dicer
RNA-mediated gene silencing
Cerebellar granule
Golgi-reticulum

ABSTRACT

RNA-mediated gene silencing is recently emerged as a fundamental mechanism of regulation of gene expression in many organisms and tissues, with special emphasis with respect to the nervous system. With the aim to study the components of RNA silencing machinery, we have investigated the expression profile and localization of dicer protein RNase III endonuclease in cultures of post-mitotic neurons. Dicer catalyzes the processing of double-stranded RNAs (dsRNAs) into ≈21–25 nucleotide-long small interfering (si)RNAs and micro (mi)RNAs, and it represents an essential step in the biogenesis of these small non-coding RNA molecules. We show that in rat primary neurons dicer is localized in the somatodendritic compartment, at the Golgi-reticulum area network level. This peculiar distribution was altered by brefeldin A treatment. Moreover the Golgi-reticulum dicer signal was observed also in primary astroglial cells. In addiction dicer was observed to be regulated during the embryogenesis and development in several tissues. In fact its expression is developmentally regulated in cultured cerebellar granule neurons. This is the first study in which dicer is shown preferentially distributed in the Golgi-reticulum area in post-mitotic terminally differentiated neuronal and glial cells and that its profile is modulated during maturation and development of in vitro cultured cerebellar granule neurons.

1. Introduction

The RNase III endonuclease dicer enzyme catalyzes the processing of double-stranded RNAs (dsRNAs) into ≈22 nucleotide-long small interfering (si)RNAs and micro (mi) RNAs. Long primary miRNAs (pri-pre-miRNA) are processed into hairpins of 60–70 nucleotides (pre-miRNA) and exported to the cytoplasm and processed to ≈21–25 nucleotide dsRNA molecules (miRNA) (Gregory et al., 2004). This cleavage is an essential step in the biogenesis of these small non-coding RNA molecules, which operate as guides for RISC (RNA-Induced Silencing Complex) to cleave a target messenger

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Abbreviations: miRNAs, microRNAs; siRNAs, small interfering RNAs; dsRNAs, double-stranded RNAs; RNAi, RNA interference; CGNs, cerebellar granule neurons; FBS, fetal bovine serum; BME, basal medium eagle; AraC, cytosine-β-D-arabinofuranoside; BFA, brefeldin A; CHX, cycloheximide; PURO, puromycin; TG, thapsigargin; WGA, wheat germ agglutinin; GFAP, glial fibrillary acid protein; GalC, galactocerebroside; ER, endoplasmic reticulum; DIV, days in vitro

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doi:10.1016/j.brainres.2007.07.088

RNA in case of a perfect complementarity (siRNA) or to block the target mRNA translation (miRNA) when there is an imperfect complementarity between miRNAs and the target gene. In mammalian cells the repression of translation by miRNA consists in a sequence specific pairing with 3′UTR of the mRNA target (Bartel, 2004). The role of RNA gene silencing in the nervous tissues is under active investigation since several experimental evidences indicate a fundamental role of the small RNAs in nervous system (Mehler and Mattick, 2006; Cao et al., 2006; Kosik, 2006;). Several mouse brain-specific miRNAs were cloned (Krichevsky et al., 2003; Kim et al., 2004; Sempere et al., 2004) and associated to neuronal cell differentiation (Smirnova et al., 2005). Specific microRNA subsets were expressed in specific brain area and in neuronal and glial cell subtypes (Krichevsky et al., 2003; Kosik and Krichevsky, 2005; Krichevsky et al., 2006; Smirnova et al., 2005). Moreover the miRNAs are involved in synaptic plasticity (Schratt et al., 2006) and in neurological Tourette's syndrome (Abelson et al., 2005). Dicer is a member of the ribonuclease III superfamily of double-stranded (ds)RNA-specific endo-ribonucleases which is expressed in all eukaryotes, encoded by a single-copy gene and it is essential in mouse (Bernstein et al., 2003) and for brain morphogenesis in zebrafish (Giraldez et al., 2005). It is a multidomain ribonuclease protein with a molecular weight of ≈200 kDa, which interacts with several members of PAZ and PIWI domain (PPD) proteins and other proteins as heat shock protein 90 (HSP90), fragile X mental retardation protein (FMRP) and TRBP (Kolb et al., 2005; Chendrimada et al., 2005). Although a growing body of evidence is related to the expression of specific miRNAs in several cells, little is known about the regulation and sub-cellular localization of the RNAi machinery in post-mitotic neuronal cultures in particular during in vitro development and maturation. It was recently shown that dicer accumulates in dendritic spines and postsynaptic densities of mouse brain extracts and that it is activated by intracellular calcium (Lugli et al., 2005). In mouse and rat lens the dicer protein is present during late em-

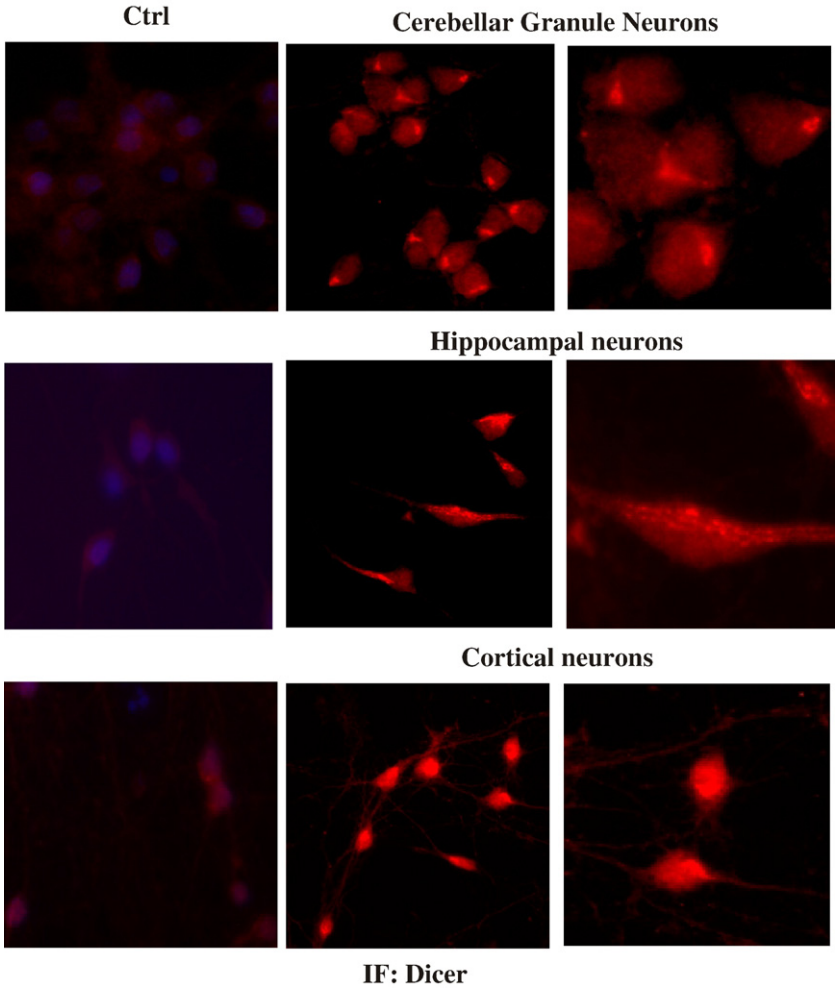

Fig. 1 – Cellular localization of dicer in post-mitotic neurons. Indirect immunofluorescence of in vitro cultured rat cerebellar granules, hippocampal and cortical neurons both fixed and processed with anti-dicer rabbit polyclonal antibody (1:100). From the left, first column: the control staining (only secondary antibody and nuclei with Hoechst); second column: it is notable that the dicer signal diffusion is dependent on neuronal cytoplasm: compact in CGNs, diffused in proximal and distal dendrites in hippocampal neurons and diffused in cortical neurons; third column represents an enlargement for every neuronal type cultured, which clearly show the cellular distribution of dicer in several neuronal types.