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Production of Prion Protein (PrP^C) in Transfected Murine Erythroleukemia (MEL) and Neuronal N2a Cells Overexpressing PrP RNA Transcripts

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S u m m a r y. In this study we developed stably transfected murine erythroleukemia (MEL) and neuronal N2a cells expressing the Prn-p gene at high levels. Northern blot hybridization and Western blotting revealed that stably transfected MEL and N2a cells are capable of producing PrP^C at different quantities. These data indicate: (a) Cells of hematopoietic origin like MEL are also capable of expressing PrP^C as the cells of neuronal origin, (b) PrP^C can be easily produced and studied in cultures of mouse hematopoietic and neuronal cells, (c) Stably transfected N2a and MEL cells can be used as models/systems for screening of potential inhibitors for the conversion of PrP^C to its pathological isoform PrP^{Sc} in culture.

INTRODUCTION

Prion protein (PrP^C) has been established as the major component of the prion infectious agent that causes Transmissible Spongiform Encephalopathies (TSEs). According to the *protein only hypothesis* PrP^C is converted into a pathological isoform termed PrP^{Sc} that is considered responsible for brain damage in prion infected animals (1). PrP^C is a glycosylated plasma membrane protein with an exofacial orientation and a physiological role that still remains unknown. Earlier studies have indicated that PrP^C protein is not produced in erythrocytes and granulocytes (2).

RESULTS AND DISCUSSION

In an effort to investigate the role of expression of prion gene (*Prn-p*) in mouse erythroleukemia (MEL) and mouse neuronal N2a cells, we stably transfected both cell lines with pcDNA 3.1 eukaryotic expression vector bearing the entire mouse ORF of the *Prn-p* gene (3). The selected clones were evaluated by Northern blot hybridization and Western blotting for PrP RNA transcripts and accumulation of PrP^C glycosylated isoforms, respectively. The detection of PrP protein was carried out with the use of 4F2 monoclonal anti-body used for immunoprecipitation of soluble extracts derived from cultured cells (4). The results obtained are illustrated in Figure 1.

Although MEL cells express PrP^C at hardly detectable levels, stable transfection with *Prn-p* gene has generated clones producing PrP protein in easily detected quantities. These data indicate that hematopoietic cells are capable to synthetize PrP^C protein, an event observed for the first time.

Interestingly, stably transfected N2a cells (clone 13N2a) produced PrP^C at quantities comparable to those seen in prion infected brain tissue derived from sheep. The amount of PrP^C expression in N2a parental cells and clones were far greater to that seen in parental and transfected MEL cells, indicating that PrP^C is produced at higher level in neuronal than in hematopoietic cells.

These results indicate that PrP^C can be easily produced in cultured cells overexpressing *Prn-p* gene. Additionally, recombinant PrP protein can be used as a substrate for *in vitro* conversion of PrP^C to PrP^{SC}, whereas the two cell systems can be useful for studying biosynthesis of PrP^C and its conversion into PrP^{Sc} in culture. Finally, such model systems can be used for screening potential inhibitors of PrP^C to its pathological isoform PrP^{Sc}.

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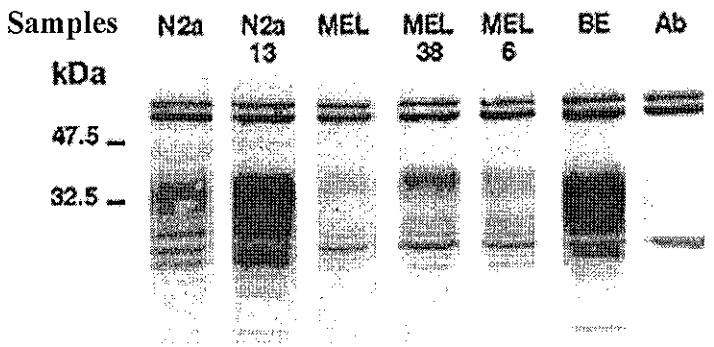

Figure 1. Assessment of PrP protein level in MEL, N2a and stably transformed clones. Cytoplasmic extracts (3mg) of N2a, MEL and clones 13N2a, 38MEL, 6MEL were immunoprecipitated with 4F2 monoclonal antibody (1:150). BE: Total protein extract (3mg) derived from infected sheep brain was immunoprecipitated with the same antibody and used as a positive marker. Ab: 4F2 antibody.