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Point mutation in the *NF2* gene of HEI-193 human schwannoma cells results in the expression of a merlin isoform with attenuated growth suppressive activity

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Abstract

Neurofibromatosis type 2 (NF2) is a genetic disorder characterized by the formation of bilateral schwannomas of the eighth cranial nerve. Although the protein product of the *NF2* gene (merlin) is a classical tumor suppressor, the mechanism by which merlin suppresses cell proliferation is not fully understood. The availability of isolated tumor cells would facilitate a better understanding of the molecular function of merlin, but primary schwannoma cells obtained from patients grow slowly and do not yield adequate numbers for biochemical analysis. In this study, we have examined the *NF2* mutation in HEI-193 cells, an immortalized cell line derived from the schwannoma of an NF2 patient. Previous work showed that the *NF2* mutation in HEI-193 cells causes a splicing defect in the *NF2* transcript. We have confirmed this result and further identified the resultant protein product as an isoform of merlin previously designated as isoform 3. The level of isoform 3 proteins in HEI-193 cells is comparable to the levels of merlin isoforms 1 and 2 in normal human Schwann cells and several other immortalized cell lines. In contrast to many mutant forms of merlin, isoform 3 is as resistant to proteasomal degradation as isoforms 1 and 2 and can interact with each of these isoforms *in vivo*. Cell proliferation assays showed that, in $NF2^{-/-}$ mouse embryonic fibroblasts, exogenously expressed merlin isoform 3 does exhibit growth suppressive activity although it is significantly lower than that of identically expressed merlin isoform 1. These results indicate that, although HEI-193 cells have undetectable levels of merlin isoforms 1 and 2, they are, in fact, not a merlin-null model because they express the moderately active growth suppressive merlin isoform 3.

Keywords: Merlin; Schwannomin; NF2; ERM proteins

1. Introduction

Neurofibromatosis type 2 (NF2) is an inherited disorder that predisposes patients to the formation of bilateral schwannomas of the eighth cranial nerve and an increased propensity for the formation of meningioma and spinal ependymoma [1]. The gene responsible for NF2 was cloned in 1993 [2,3]. The *NF2* transcript can be alternatively spliced to form many *NF2* variants [4,5], the most abundant of which are isoforms 1 and 2, which comprise approximately 90% of the mature *NF2* transcript within cells ([6], see Fig. 1). Only isoform 1 has been shown to suppress cell growth in cell model systems [7].

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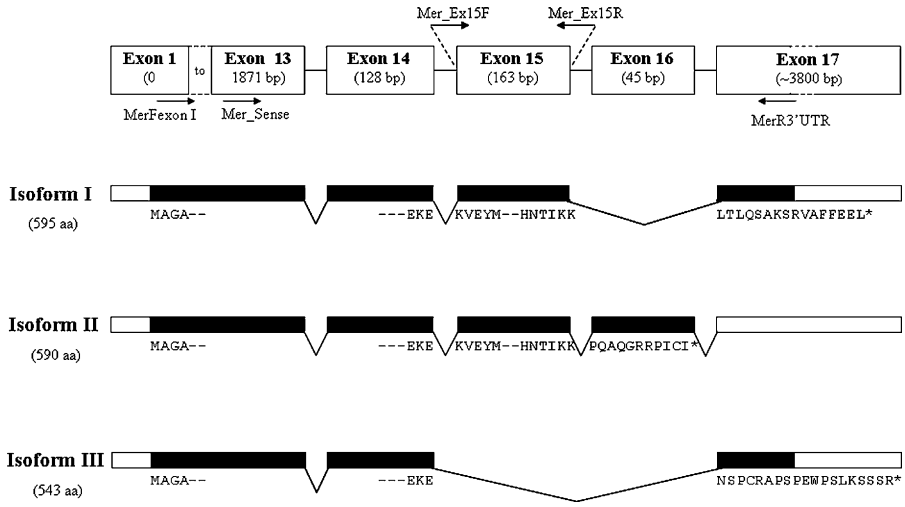

Fig. 1. Schematic of *NF2* isoforms. *NF2* isoforms 1–3 are diagrammatically represented and aligned with the *NF2* gene to show the contribution of the different exons to the resultant mRNA structure and protein sequence. *NF2* isoform 2 differs from isoform 1 by the addition of exon 16, resulting in the substitution of the last 16 amino acids of isoform 1 with 11 different ones. Isoform 3 lacks both exon 15 and 16, resulting in a protein C-terminus different from both merlin 1 and 2. The arrows represent the relative positions of primers used for PCR and RT-PCR analysis. The open rectangles represent the non-translated region whereas the black rectangles represent the translated region of the mRNA. The asterisks denote positions of stop codons.

The mechanism by which merlin regulates cell proliferation is not fully understood. Merlin can block Rac-mediated signaling [8], perhaps directly through inhibition of Pak activity [9]. Consistent with this notion, tumor-derived *NF2*$^{-/-}$ schwannoma cells display aberrant membrane ruffling that is characteristic of hyperactivated Rac [10]. This phenotype can be corrected by addition of dominant negative Rac [10] or wild type merlin [11], suggesting that loss of merlin in schwannoma cells may lead to cellular hypertrophy as a result of elevated Rac signaling.

Merlin also is known to play an important role in contact-dependent growth arrest [12,13]. Embryonic fibroblasts derived from *Nf2*-deficient mice continue to grow beyond confluency, and their inability to form stable adherens junctions appears to be at least partially responsible for this aberrant phenotype [13].

Although studies using primary schwannoma cells have yielded significant information, their use also has significant drawbacks. Typical tumor specimens yield extremely low numbers of cells and the cells grow very slowly in culture. In addition, primary schwannoma cells are refractory to gene transfection, making mechanistic studies difficult. Thus, the inherent difficulties in obtaining, maintaining, and manipulating primary schwannoma cells led us to evaluate the immortalized schwannoma cell line HEI-193 as an alternative cell system with which to study merlin function.

The HEI-193 cell line was derived from an NF2 patient with spontaneous bilateral vestibular schwannomas as well as a history of meningioma [14]. The schwannomas were surgically removed and their constituent cells subsequently immortalized using the human papilloma virus E6–E7 genes [15]. Previous work detected a germ-line mutation in the *NF2* gene at −1 position of the intron 14/exon 15 borders. This mutation is predicted to destroy the donor sequence of exon 15 and result in exon skipping [16]. The presence of a shorter *NF2* transcript in HEI-193 cells was confirmed by RT-PCR [15]. However, the molecular alterations in the *NF2* transcript and the encoded merlin protein were not fully described.

In this paper we report that the merlin protein expressed in HEI-193 cells has amino acid sequence identical to that of a splice variant previously designated as isoform 3 [17]. This isoform was first described in a family with a history of a mild form of NF2 and was shown to arise because of an A → T mutation within the *NF2* gene at the +3 position of the donor site of intron 15 [17]. Interestingly, isoforms 1–3 are simultaneously and equivalently expressed both at the RNA and protein levels in fibroblasts derived from this family, but in schwannoma cells only isoform 3 are expressed [17]. Based on the mild nature of the NF2 disease phenotype seen in this family, the authors of this study concluded that merlin isoform 3 retained mild tumor suppressive activity.

Here we present evidence that HEI-193 cells express merlin isoform 3 with no detectable isoform 1 or 2. The level of merlin isoform 3 in HEI-193 cells is comparable