Neuronal-specific Missplicing in Familial Dysautonomia

George J.Siegel

Familial dysautonomia (FD, also known as Riley–Day syndrome) is a disease consisting of autonomic and sensory neuropathy inherited as an autosomal recessive trait, present almost exclusively in Ashkenazi Jews with an incidence of 1/3600 live births. The carrier frequency in this population is 1/30. The disease is manifested at birth with poor sucking, failure to thrive, unexplained bouts of fever, and episodes of lung infections. In the growing child, there are manifestations of autonomic nervous system abnormalities such as lack of tears, defective temperature control, labile blood pressure, postural hypotension, excessive sweating, GI symptoms, absence of tongue fungiform papillae and autonomic crises with sweating, hypertension and vomiting. There is marked impairment of pain and temperature sensation. There is poor development and progressive degeneration of the sensory and autonomic nervous system. Nerve biopsies reveal decreases in the numbers of small myelinated and unmyelinated fibers, which explains the impairment of pain and temperature sensory function. Autopsy studies show diminished numbers of neurons in sympathetic and parasympathetic ganglia, in intermediolateral gray column, and, to a lesser extent, diminished neurons in sensory dorsal root ganglia (Pearson & Pytel, 1978).

Serum levels of dopamine beta hydroxylase, which hydroxylates dopamine to form norepinephrine (NE) in presynaptic NE-containing vesicles, are significantly reduced. This reduction is diagnostic. Patients excrete in their urine increased amounts of homovanillic acid (HVA), which is a dopamine metabolic product, and decreased amounts of vanillylmandelic acid (VMA) and methoxyhydroxyphenylglycol (MHPG), which are norepinephrine metabolic products [see text in this chapter]. Also, reduced levels of monoamine oxidase A have been

found in cells and tissues of FD patients (Rubin & Anderson, 2008). These biochemical findings are consistent with decreased NE-containing terminals and NE synthesis. Abrupt uncompensated changes in levels of either dopamine or NE could be involved in hypertensive crises.

The gene mutated in FD (*IKBKAP*) encodes the IKB kinase-complex associated protein (IKAP/hELP1) which is the human homologue of the yeast ELP 1 protein (Blumenfeld et al., 1993). The latter protein is part of the RNA polymerase II–associated elongator complex, but it is not known whether hELP1 serves the identical function. Results from various experimental models implicate its function in both transcription and translation (Svejstrup, 2007).

The mutation found in 99.5% of FD patients is a T-to-C change in base pair 6 of the 5'-splice donor site on intron 20, resulting in skipping of exon 20 and causing a frameshift (with exon 19 spliced to exon 21), therefore producing a truncated protein. However, this deletion of exon 20 is tissue specific. Lymphoblast RNA from FD patients showed normal length RNA containing exon 20. RNA extracted from postmortem brain stem and temporal lobe samples showed complete absence of exon 20 from the IKBKAP mRNA. Fibroblast lines from FD patents homozygous for the mutation yielded variable results: some lines displayed about equal amounts of wild-type and mutant-type mRNAs, whereas others displayed primarily wild type. The absence of exon 20 in the brain samples of RNA together with the preponderance of wild-type RNA or variable proportions of mutation type to wild type in lymphoblast and fibroblast cell lines, all from homozygous FD patients, has led to the hypothesis that the efficiency of splicing between exon 20 and 21 in the presence of the T to C mutation at base pair 6 at the slice donor site is reduced in a tissue specific manner, and that the autonomic and sensory neuronal systems have a particularly greater sensitivity to this inefficiency. The mutation does not completely abolish the splicing of exon 20 to 21 but weakens it. The degree of weakening may,

of course, depend on other tissue-specific factors (Blumenfeld et al., 1993; Slaugenhaupt & Gusella, 2002).

The creation of a transgenic mouse line harboring the human FD-*IKBKAP* gene shows that the presence of the FD mutation causes missplicing of human *IKBKAP* in mice and that the efficiency of exon 20 inclusion varies in a tissue-specific manner that closely models that seen in FD patients. Additionally, this study showed in tissue culture experiments that missplicing of human *IKBKAP* in mouse cells can be corrected by kinetin treatment, demonstrating conservation of cellular factors required for kinetin activity (Hims et al., 2007).

It is thought that the IKAP/hELP1 functions in general gene-activation mechanisms. The FD disease may be caused by aberrant expression of genes crucial to the development of the sensory and autonomic nervous systems, secondary to the loss of a completely functional IKAP/hELP1 protein in specific tissues. However, the pathogenic mechanism for the FD phenotype is not known (Slaugenhaupt & Gusella, 2002).

A microarray expression study using RNA extracted from postmortem cerebrum of an 11-year-old male and a 47-year-old female patient with FD revealed no genes upregulated but a twofold decrease in expression of 25 genes in comparison to results from normal age- and gender-matched control samples. Of these, 13 are known to be involved in oligodendrocyte differentiation and/or myelin formation. Their downregulation was confirmed by PCR and protein analyses. These data support a view that IKAP/hELP1 controls a complex process responsible for axon development and myelination in the CNS and PNS. However, many questions remain for investigation (Cheishvili et al., 2007). A study of genes affected by IKAP/hELP1 in HeLa cells transfected with RNAi oligomers that target the IKAP/hELP1 transcript disclosed about 100 genes that were significantly downregulated, while about 15 genes

were upregulated in a microarray analysis of total extracted mRNA. It was reported that 15% of the 100 downregulated genes encode proteins involved in regulating the actin cytoskeleton, cell motility and migration. Moreover, it was shown that decreases in levels of IKAP/hELP1, in neuronally derived and other cell types, did in fact result in migration defects. It was proposed that reduced motility of neuronal-derived cells may be relevant to the neurodevelopmental disorder in FD (Close et al., 2006). Another study utilizing RNAi with primary neuronal cells also revealed that cells expressing very low amounts of IKAP displayed significant defects in migration and adhesion and that these defects were associated with the inability of filamin A to localize at leading edges of migrating cells and with disorganized actin cytoskeleton. These defects could be rescued by co-expression of wild-type IKAP but not co-expression of the truncated FD-IKAP (Naumanen et al., 2008).

The fact of tissue-specific missplicing of the *IKBKAP* transcript is a platform from which to initiate investigation of potential therapeutic or preventive strategies. Potential strategies for therapy, such as kinetin, may be tested in the transgenic mouse model mentioned above (Hims et al., 2007). Kinetin was administered orally for eight days to 29 healthy carriers of the mutated gene, which resulted in elevations of *IKBKAP* mRNA in leukocytes, thus indicating (1) a potential therapeutic or preventive effect in FD and (2) that splicing disorders might be modulated by pharmacologic means (Gold-von Simson et al., 2009).

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