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Ligase IV syndrome

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ABSTRACT

Ligase IV (LIG4) syndrome belongs to the group of hereditary disorders associated with impaired DNA damage response mechanisms. Subjects affected with this rare autosomal recessive disease exhibit microcephaly, unusual facial features, growth retardation, developmental delay, skin anomalies, and are typically pancytopenic. The disease is characterized by pronounced radiosensitivity, genome instability, malignancy, immunodeficiency, and bone marrow abnormalities. LIG4 syndrome results from mutations in the DNA ligase IV gene encoding an enzyme that plays a pivotal role in repairing double strand DNA breaks and V(D)J recombination. Since LIG4 null-mutant mice are embryonic lethal and biallelic null mutations have not been described to date in LIG4-deficient patients, viability of the DNA ligase IV deficiency syndrome appears to require at least one allele with a hypomorphic mutation. Mutations R278H, Q280R, H282L, M249E located in the vicinity of the active site are typical hypomorphic because they do not affect ligase expression and retain residual albeit reduced activity of the enzyme at levels of 5–10% of that for the wild-type ligase. Carriers heterozygous for those mutations usually develop moderate defects in V(D)J recombination, mild immune abnormalities and malignancy. In contrast, mutations resided in OBD, i.e. in the C-terminal subdomain of the catalytic domain, and in XRCC4-binding domain more dramatically inhibit the ligase function and also greatly decrease its expression. A truncating mutation R580X and a frameshift mutation K424FS resulting in loss of the C-terminal XRCC4-binding domain have deleterious effect on both expression and function of LIG4 and represent a null allele.

1. Ligase IV syndrome: clinical outcomes

Ligase IV (LIG4) syndrome (MIM 606593) belongs to the group of hereditary disorders associated with impaired DNA damage response mechanisms. Subjects affected with this rare autosomal recessive disease exhibit microcephaly, unusual facial features, growth retardation, developmental delay, skin anomalies, and are typically pancytopenic [1]. The disease is characterized by pronounced radiosensitivity, genome instability, malignancy, immunodeficiency, and bone marrow abnormalities. LIG4 syndrome shares similar clinical phenotypes with other rare DNA damage response diseases such as Seckel syndrome, Nijmegen breakage syndrome (NBS), and Fanconi anemia (Table 1) [2]. However, unlike NBS cell lines, cells derived from LIG4 patients showed normal cell cycle checkpoint responses but impaired DNA double strand breaks (DSB) rejoining. In T and B-cell precursors of LIG4 syndrome patients, an unusual V(D)J recombination phenotype was observed involving a decrease in rejoining frequency coupled with elevated imprecision at signal junctions [1].

2. DNA ligase IV is a component of the non-homologous end-joining pathway of DNA repair

LIG4 syndrome arises from mutations in the *LIG4* gene encoding DNA ligase IV (MIM 601837, EC 6.5.1.1), a component of the non-homologous end-joining (NHEJ) machinery, which represents a major mechanism of repair of DSBs in mammals [4]. DSBs are generally induced by ionizing radiation but also generated during V(D)J recombination, the essential rejoining process that serves to rearrange the variable, diversity and joining segments in T and B cell development [5].

NHEJ repair is a multistep process that requires the involvement of several proteins (Fig. 1). Briefly, the heterodimeric Ku70/80 protein binds to the DNA ends in a site where a DSB occurs. DNA-bound Ku serves to recruit the compex between Artemis and DNA-dependent protein kinase catalytic subunit (DNA–PKcs) and then activate its kinase activity as well as to attract binding the

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Table 1
Clinical overlaps between LIG4 syndrome, Nijmegen breakage syndrome, Fancony anemia, and Seckel syndrome.

Clinical phenotype	Nijmegen breakage syndrome[a]	Fanconi anemia[a]	Seckel Syndrome[a]	LIG4 syndrome
Microcephaly	+	+	+	+
External ear abnormalities	+	+	+	–
Long/large nose	+	–	+	+
Polydactyly	+	+	–	–
Sindactyly	+	–	–	–
Skin abnormalities	+	+	+	–
Mental retardation/ developmental delay	+	+	+	+
Malignancy	+	+	+	+
Recurrent infections	+	+	–	+
Pancytopenia	–	+	–	+
Genital abnormalities	+	+	+	+
Radiosensitivity	+	+	+	+
Immunodeficiency	+	+	–	+
Bone marrow abnormalities/ failure	+	+	+	+
Gene	NBS1	FANC	ATR	LIG4

[a] Features obtained from The London Dysmorphology Database [3].

DNA ligase IV/XRCC4 complex to the DSB site. DNA–PKcs undergoes autophosphorylation and also phosphorylates Artemis, which regulates its binding to DNA, and is required for its function in DSB rejoining [6]. The phosphorylation permits the Artemis/DNA–PKcs complex to function as an endonuclease allowing it to cleave both 5′ and 3′ overhangs of any length [7].The DNA ligase IV/XRCC4 complex exists endogenously in a pre-adenylated form. Recruitment of the DNA ligase IV/XRCC4 complex to DNA causes inward translocation of Ku allowing the AMP moiety of the ligase–adenylate complex to transiently attach to the DNA end promoting ligation [8].

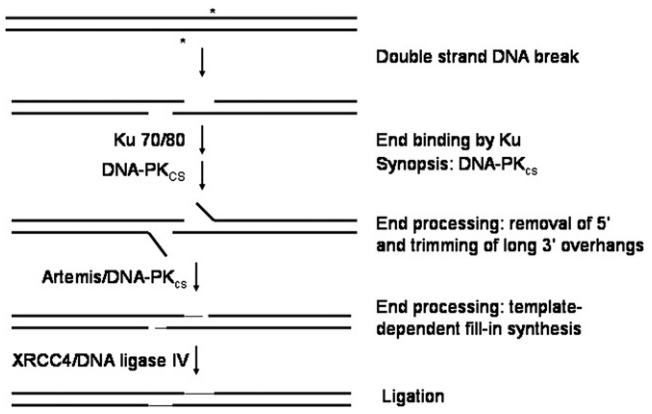

Fig. 1. The mechanism of the vertebrate non-homologous DNA end joining (NHEJ) pathway. A double strand DNA break (DSB) formation induces Ku70/80 heterodimer binding to DNA ends in the DSB site. End binding by Ku allows holding of DNA ends in proximity. This step can be referred to as synapsis. Though Ku alone may not be able to achieve synapsis, there is some evidence that DNA-dependent protein kinase catalytic subunit (DNA–PKcs) is capable of noncovalently holding on to each of the two DNA ends. For incompatible DNA ends generally generated by ionizing radiation, there is often need for nucleolytic processing. If so, Artemis:DNA–PKcs are likely to carry out most, and perhaps all, such processing. For only a subset of DNA ends, end alignment can occur at chance sites of terminal microhomology of 1–4 nucleotides. End processing not only includes nucleolytic removal of sections of the DNA termini, but also the filling in of gaps by polymerases. Ligation is the final step, and it requires a ligatable nick on each strand. Ligation in NHEJ is performed by the XRCC4:DNA ligase IV complex. Rejoining of a simple pair of DNA ends (blunt or compatible), may not require a nuclease or a polymerase.

3. DNA ligase IV: structure and function

The *LIG4* gene was mapped to chromosome 13q33–q34 [9]. The gene encompassing 10.9 kb consists of 2 exons and one intron. A cDNA encoding a LIG4 polypeptide of 911 amino acids with a predicted mass of 96 kDa was first isolated from HeLa cells [9].

DNA ligase IV has a complex structure formed by several domains (Fig. 2A). The enzyme contains a conserved ligase domain at its N-terminus and a tandem BRCT domain at its C-terminus [7]. Interaction with XRCC4 requires the region between the BRCT domains and likely part of the BRCT domain [10]. The first step of ligation involves formation of a covalent AMP-enzyme intermediate with AMP being attached to the enzyme via a highly conserved lysine residue (K273). The second step involves formation of a DNA–adenylate complex finally followed by rejoining. In all ATP-dependent DNA ligases, the catalytic domain has a modular structure of two domains, an adenylation domain (AdD) and an oligo-binding domain (OBD) [11]. The larger eukaryotic ligases such as ligase I and ligase IV also possess an additional N-terminal DNA-binding domain (DBD) that is required for efficient ligation and enables these ligases to encircle DNA [12]. Six conserved motifs and designated motifs I, III, IIIa, IV, V, and VI have been identified among covalent nucleotide transferases, of which five are found in the AdD. Motifs I, III, IIIa, IV, and V are essential for ATP binding and autoadenylation reaction. Motif I, encompassing the conserved lysine residue, forms the active site loop of the enzyme and constitutes part of the ATP binding pocket. Based on crystal structures of a number of DNA ligase complexes, it has been proposed that ligases undergo profound conformational changes upon ATP binding and/or DNA-binding [13]. This is exemplified by the rotation of the OBD. Motif VI lies within the OBD, distant from the active site on AdD [14]. However, upon ATP-binding this face of OBD moves towards the active site and residues including those from motif VI participate in the adenylation reaction [15]. Subsequently, the OBD moves away from the active site and swivels around placing motif VI far from the AdD orientating the DNA-binding surface of OBD towards the now adenylated AdD [16]. This switching is essential for the catalytic cycle as it most likely prevents the formation of non-productive complexes between non-adenylated ligase and unnicked/unbroken DNA.

Recently, Marchetti et al. [17] identified in ligase IV a highly conserved motif termed motif Va. The motif Va, which consists of 9 amino acids (codons 468–476), forms a loop-like structure on the surface of OBD and makes direct contact with the DNA. Experiments with genetically engineered mutants showed the involvement of two glycine residues at positions 468 and 469 in adenylate complex formation in vitro, whereas the remaining residues in the motif Va did not play critical roles in DNA ligase IV–adenylate complex formation but significantly impaired the double-stranded ligation activity [17].

4. Animal models with genetic defects in *Lig4*

Animal cell lines lacking one or both copies of the *Lig4* gene showed marked sensitivity to X-rays and DNA-damaging agents due to defective NHEJ DSB repair [18,19]. Knocking out of *Lig4* in mice is deadly causing embryonic lethality from massive neuronal apoptosis, arrested lymphogenesis, and multiple cellular defects [20]. The animal death could result from the p53-directed apoptosis caused by unrepaired DNA damage since mice with double deficiency for *Lig4*/*p53* was shown to escape from embryonic neuronal apoptosis but not from defects in lymphocyte development [20]. This suggests for a particular and non-redundant role of DNA ligase IV in V(D)J recombination in lymphocyte precursors [21]. The *Rad54*/*Lig4* double-mutant mice showed serious defects in cell