A novel missense mutation of the CYLD gene identified in a Hungarian family with Brooke–Spiegler syndrome

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Abstract: Brooke–Spiegler syndrome (BSS; OMIM 605041) is an autosomal dominant disease characterized by skin appendage tumors due to mutations in the cylindromatosis gene (CYLD). We investigated a Hungarian BSS pedigree with two affected members, father and daughter. Direct sequencing demonstrated a novel missense mutation (c.2613C>G; p.His871Gln) in exon 19 within the ubiquitin-specific protease domain of the encoded protein. We performed preliminary analysis to reveal the functional role of this novel mutation. Our data suggest that this novel CYLD mutation leads to increased ubiquitination of NEMO through influencing deubiquitinating activity of the CYLD protein and thus may result in enhanced NF-κB signalling.

Key words: appendage tumor, Brooke–Spiegler syndrome, CYLD gene, NEMO protein, ubiquitination

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Background
Brooke–Spiegler syndrome (BSS; OMIM 605041) has been described as an autosomal dominant disease characterized by the development of a wide variety of skin appendage tumors, such as cylindromas, trichoepitheliomas and spiradenomas (1,2).

The gene responsible for BSS, the cylindromatosis gene (CYLD), is localized at 16q12-q13 (2). So far 86 mutations have been identified, mainly at the 3’ end of the CYLD gene (exons 8–20; 3–9). These mutations have been identified in patients with phenotypic features of either BSS, familial cylindromatosis (OMIM 132700) or multiple familial trichoepithelioma type 1 (OMIM 601606; 10).

The CYLD gene codes for a cysteine protease type deubiquitinase, which directly interacts with and deubiquinates TRAF2, TRAF6 (TNF receptor-associated factors) and NEMO (IKBKG; an inhibitor of the kappa light polypeptide gene enhancer in B cells, kinase gamma; 11–13). Thus, CYLD attenuates TNF-α-induced classical NF-κB signalling leading to programmed cell death (14,15). Reduced CYLD activity results in an elevated activity of NF-κB and hence increased resistance to apoptosis and carcinogenesis (14,15). Through the deubiquitination of the dishevelled protein, CYLD can also influence WNT/β-catenin signalling (16).

Questions addressed
This study reports a novel missense mutation on the CYLD gene in a Hungarian family with BSS, which by influencing the deubiquitination activity of CYLD leads to increased ubiquitination of NEMO.

Experimental design
A pedigree from the Southern part of Hungary (Szeged region) affected by BSS was investigated. The pedigree has two affected members, father and daughter. Direct sequencing demonstrated a novel missense mutation (c.2613C>G; p.His871Gln) in exon 19 within the ubiquitin-specific protease domain of the encoded protein. We performed preliminary analysis to reveal the functional role of this novel mutation. Our data suggest that this novel CYLD mutation leads to increased ubiquitination of NEMO through influencing deubiquitinating activity of the CYLD protein and thus may result in enhanced NF-κB signalling.

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The activity of CYLD protein in vitro, demonstrated by its reduced enzymatic activity (14).

To reveal the function of this novel mutation, we studied the known CYLD-regulated pathways. As CYLD is known to directly interact with the NF-κB signalling NEMO protein, the expression of NEMO was determined and found to be significantly reduced in the CD4+ T lymphocytes of the patients compared to healthy controls (data not shown). Our preliminary data suggest that decreased NEMO expression is associated with its altered deubiquitination; NEMO immunoprecipitated from fibroblasts carrying the CYLD mutation demonstrated significantly higher ubiquitination than NEMO immunoprecipitated from control fibroblasts (Figure S1).

As it is known that CYLD protein is expressed in nearly every human tissue with the highest expression level in the CD4+ and CD8+ lymphocytes and CYLD deficiency can lead to defects in T-cell maturation and B-cell responses (17–25), however, others reported on normal T- or B-cell phenotype (24,26,27). We characterized peripheral blood lymphocytes in patients with BSS, but only minor alterations were observed (data not shown).

We also investigated whether the newly identified mutation affects the WNT/β-catenin pathway as it has been recently suggested as one of the functions of CYLD protein (16). For that, we performed immunohistochemical detection of the translocation of β-catenin in the tissue samples of patient I-1, but found no alteration in the intracellular distribution of β-catenin (data not shown).

We identified here a novel mutation (c.2613C>G p.His871Gln) of CYLD in a Hungarian BSS pedigree. Identification of the underlying mutation may have a considerable impact on family planning because it offers the possibility of prenatal mutation screening. Our functional studies revealed a decreased NEMO protein level in the CD4+ T lymphocytes of the patients compared to healthy controls. As in the literature, there is evidence that CYLD can decrease the ubiquitination of NEMO, but there are no data suggesting that CYLD can influence the expression level of the NEMO protein, we have measured its ubiquitination after immunoprecipitation and found an increased level of ubiquitination of the NEMO protein in fibroblasts carrying the novel CYLD mutation. However, it is well known that CYLD removes only the Lys63-linked polyubiquitin chain taking part in the proteosomal-independent cellular processes (11), and it has been recently reported that 26S proteasome can degrade Lys63-linked ubiquitin substrates (27) raising the possibility that the Lys63-linked ones can also serve as a targeting signal for proteasomal degradation.

Based on our results, we suppose that this novel mutation through the increased ubiquitination of NEMO leads to decreased NEMO expression and as a consequence may influence the NF-κB pathway. Further studies are needed to elucidate the exact mechanism of the development of BSS symptoms. As BSS displays only skin tumors, and mainly appendageal tumor formations, it may be an appropriate model for the development of novel gene therapy methods.

Acknowledgements

Attenuation of contact hypersensitivity by cell-permeable heat shock protein 70 in BALB/c mouse model

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Abstract: In contact hypersensitivity (CHS), multiple cells, inflammatory mediators and cytokines are known to be involved in the regulation of the immune response. Previously, we revealed the reactive oxygen species generation by 2, 4, 6-trinitrobenzene sulphonic acid (TNBS) in vivo, followed by heat shock protein 70 (Hsp70) carbonylation and the exogenous antioxidant role of cell-permeable Hsp70. Here, we demonstrate the role of Hsp70 using cell-permeable Hsp70 in the mouse CHS model. Pretreatment of cell-permeable Hsp70: (i) suppressed ear swelling; (ii) down-regulated phosphorylated p38, but up-regulated phosphorylated extracellular signal-regulated kinase; (iii) increased population of CD4⁺CD25⁺Foxp3⁺ T cells; (iv) decreased secretion of tumor necrosis factor-α (TNF-α), IL-12, interferon-γ and IL-2 and (v) but up-regulated IL-4 and transforming growth factor beta (TGF-β) in the lymph nodes. In conclusion, cell-permeable Hsp70 attenuates CHS through modulation of MAPK pathway and regulation of Th1, Th2 and regulatory T cells.

Key words: allergic contact dermatitis, heat shock protein 70, inflammatory cytokines, protein transduction domain

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References


Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. NEMO was immunoprecipitated from fibroblasts of BSS patients (n = 2) and from fibroblasts of healthy controls (n = 2).

Data S1. Experimental design.

Conflict of interests

The authors have declared no conflicting interests.