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Review

The NF-κB signaling pathway in human genetic diseases

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Abstract. The nuclear factor-κB (NF-κB) signaling pathway plays a key role in inflammation, immune response, cell growth control and protection against apoptosis. Recently, it has been associated with several distinct genetic diseases that exhibit a large spectrum of dysfunction, such as skin inflammation, perturbed skin appendage development and immunodeficiencies. In this review, a summary of the pathophysiological consequences of impaired NF-κB activation in humans is provided with respect to the functions of the molecules which are mutated.

Key words. NF-κB; NEMO; Incontinentia pigmenti; Anhidrotic ectodermal dysplasia with immunodeficiency; Cylindromatosis; CYLD.

Introduction

The NF-κB signaling pathway: an overview

The nuclear factor-κB (NF-κB)/Rel family of transcription factors is composed of a set of related, evolutionarily conserved DNA-binding proteins consisting of p50, p52, RelA/p65, c-rel and RelB. In most cell types, NF-κB proteins are sequestered in the cytoplasmic compartment, associated with members of the inhibitor of κB (IκB) family (IκBα, IκBβ and IκBε). In response to multiple stimuli such as inflammatory cytokines, bacterial lipopolysaccharide (LPS), viral infection or stress IκBs are phosphorylated on two critical serine residues. This modification triggers their ubiquitination and destruction via the proteasome degradation machinery. As a consequence, free NF-κB enters the nucleus and activates transcription of a variety of genes participating in immune and inflammatory responses, cell adhesion, growth control and regulation of apoptosis [1, 2].

Except for ultraviolet (UV) light, all the NF-κB activators have been shown to target a high molecular weight (700–900 kD) IκB kinase (IKK) complex. This complex is composed of two catalytic subunits (IKK-1 or -α and IKK-2 or -β), a regulatory subunit (NEMO/IKK-γ) and probably other subunits less well characterized, such as Cdc37 and Hsp90 [3] or the recently discovered ELKS subunit [4] (fig. 1).

NEMO, which is encoded by a gene located on the X chromosome [5], is composed of several structural domains, among them two coiled-coil domains, a leucine zipper and a zinc finger (fig. 2). It is required for IKK activation in response to most NF-κB stimuli ('Canonical' pathway), as shown using mutant cell lines defective for this protein [6]. In contrast, a subset of stimuli, including BAFF or LTβ, do not require NEMO but induce IKK activation through NIK-induced IKK-1 phosphorylation ('Non canonical' pathway) [7].

Numerous questions remain regarding the exact mechanism of IKK activation and the identity of the signaling molecules which participate in the fine tuning of IKK activation and inactivation. Very recently, it was proposed that IKK activity may be turned on, at least in the canonical pathway, by a ubiquitination event not connected to proteolysis, and that this modification is negatively controlled by the CYLD deubiquitinase (see details below) [8].

Through the use of transgenic mice the various roles of NF-κB in vivo have been determined. A recurrent fea-

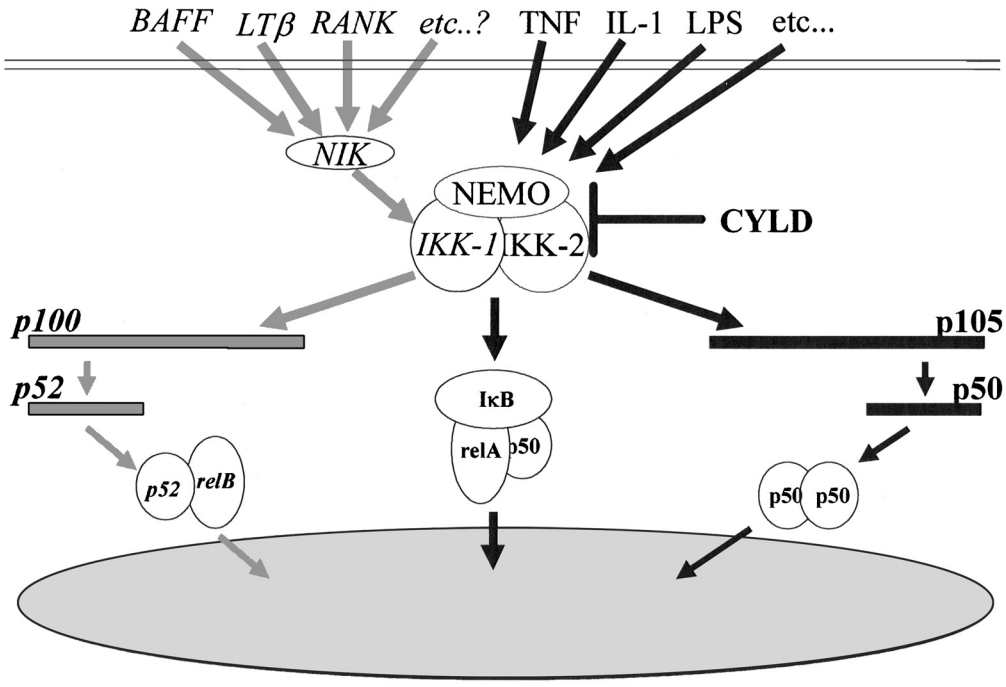

Figure 1. NF-κB activation pathways. Both the canonical (black arrows) and the noncanonical (grey arrows) pathways of IKK activation are presented. The NEMO-dependent canonical pathway induces activation of IκB-bound NF-κB species (such as relA/p50 or c-rel/p50) and processing of the p105 precursor of p50. The NEMO-independent non-canonical pathway induces processing of the p100 precursor of p52 that forms a dimer with relB. In addition to their precursor role, p100 and p105 also exhibit IκB-like properties by retaining NF-κB subunits in the cytoplasm. CYLD deubiquitinase negatively regulates IKK activation through the canonical pathway but not through the non-canonical one.

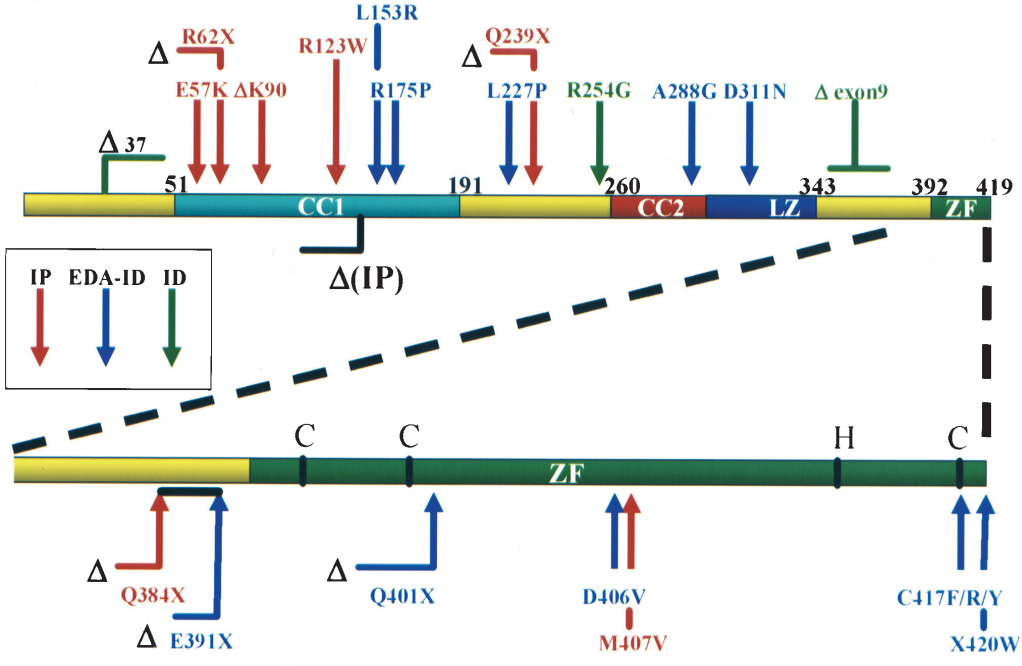

Figure 2. NEMO mutations in human pathologies. All the reported missense mutations, nonsense mutations and small deletions (Δ) associated with IP (orange arrows), EDA-ID (blue arrows) and ID (green arrows) are presented. In addition, the NEMO deletion that results from the reccurent DNA rearrangement found in IP is indicated [Δ(IP)]. To simplify the picture, frameshift mutations that lead to truncation of NEMO are not shown. Several of them affect the region around Q384/E391 (black bar) and generate either IP or EDA-ID, depending on the number of irrelevent amino acids added (see comment in the text). CC; coiled coil, LZ; leucine zipper, ZF; zinc finger.

ture of impaired NF-κB activation is embryonic lethality due to massive tumor necrosis factor α (TNF-α)-induced apoptosis occuring in the liver. This has been observed for instance for *relA* knockout [9], *ikk-2* knockout [10–12] and *nemo* knockout [13–15] (see below) mice. When less essential components of the NF-κB pathway are targeted, or when the knockout strains listed above are crossed with *tnf-r1* knockout mice to avoid premature death by liver apoptosis, many immune dysfunctions are observed, demonstrating the essential role that this pathway plays in both innate and acquired immunity [16].

In this review, I summarized what has recently been learned in the field of genetic diseases caused by NF-κB dysfunction, focusing more specifically on pathologies resulting from mutations affecting core components of the NF-κB cascade.

NEMO-related diseases

Incontinentia pigmenti

Incontinentia pigmenti (IP) (OMIM#308300) is a severe X-linked genodermatosis (frequency: 1/10,000–1/100,000) that presents exclusively in females, as male cases die in utero between the 14th and 17th weeks of pregnancy [17]. In affected females the disorder is highly variable in presentation. Typically, IP is characterized by four distinct dermatological stages. It starts within 2 weeks after birth with blisters and an inflammatory response, accompanied by a massive eosinophilic granulocyte infiltration into the epidermis (vesicular stage). Subsequently, verrucous hyperkeratotic lesions evolve and disappear over time (verrucous stage), leaving behind areas of hyperpigmentation due to melanin accumulation, that follow the lines of Blaschko (hyperpigmented stage). These lesions generally disappear by the second decade (atrophic stage), but adults may still show areas of dermal scarring. Very rare patients can reinitiate a similar sequence of events during their teenage years or adulthood, generally following an infection [18, 19].

Besides the manifestations at the epidermis level, a fraction of IP patients also suffer from ophthalmologic (abnormalities of the developing retinal vessels), odontological (missing or deformed teeth) and, in rare cases, neurological (convulsive disorders, motor or mental retardation) problems.

Another characteristic feature of IP is skewed X-inactivation that occurs in several cell types or tissues of female patients. This skewing reflects a counterselection of the mutated cells and can reach, in blood cells for instance, more than 95%. The most striking demonstration of this potent counterselection process is the report of a female infant born to a mother with IP and a father with haemophilia A, who manifested both disorders [20].

The gene responsible for IP was originally mapped to an interval of about 2 Mb distal to the colour vision locus in Xq28. The gene encoding *NEMO/IKBKG* was also located to Xq28, and this localization as well as the high sensitivity to apoptosis of IP cells suggested that it might be responsible for this pathology. Remarkably, the analysis of a large collection of patients showed that 85% of them carried the same complex rearrangement of the *NEMO* gene [21]. This rearrangement results in excision of the region between two MER67B repeat sequences located upstream of exon 4 and downstream of exon 10, respectively. It appears to involve homologous sequences of a *NEMO* pseudogene located telomeric to *NEMO*, in a reverse orientation [22]. The first three exons of *NEMO* that remain after the rearrangement produce a truncated 133-amino acid molecule, which is devoid of activity since it does not contain C-terminal sequences that connect to IKK activators. In IP patients carrying the *NEMO* rearrangement, a lack of NF-κB activation was demonstrated by studying foetus-derived primary fibroblasts: these cells are unresponsive to all tested NF-κB-activating stimuli, they do not show degradation of the IκB molecules when stimulated, and they are very sensitive to TNF-α-induced apoptosis. Most other mutations that have been described as cause of IP result in more or less severe truncations of *NEMO* [21, 23], but few missense mutations have also been identified [24].

Mouse models of IP

The phenotype of IP patients is very difficult to assess, due to the complex interplay that exists in several tissues between cells carrying a normal copy of *NEMO* and those carrying a defective one. For some unclear reasons, X inactivation skewing is less complete in the skin and selective elimination of cells bearing a mutated X chromosome only starts at birth, generating the characteristic dermatosis observed in IP patients. In addition, the nature of the signal that triggers IP cell elimination at this particular time is itself unknown. Murine models have been developped to help answering these important questions.

Nemo knockout mice have been engineered by several groups [13–15], and their phenotype appears very similar to the one of IP patients. Male mice die very early during embryogenesis (E12) from massive liver apoptosis. As stated above, a similar phenotype has also been reported for r*elA* and *ikk-2* knockout mice [9–12] but death occurs a bit later, at E15.5 and E14.5, respectively. It remains to be established whether liver apoptosis is also responsible for male lethality in IP. In contrast to males, *nemo* knockout female mice develop normally but, soon after birth, exhibit a transient dermatosis, characterized by patchy skin lesions with massive granulocyte infiltration, hyperproliferation and increased apoptosis of keratinocytes,

reminiscent of what is observed in human IP. Hyperpigmentation, a hallmark of IP due to the presence in the dermis of phagocytes containing melanosome complexes, was also observed in skin sections from *NEMO* +/– mice. In addition, extensive X-inactivation skewing in blood leukocytes, which is a major feature of IP patients, is also present in *nemo* knockout mice [15]. The only difference that is apparent between IP pathology and *nemo* knockout is the high level of mortality that occurs in female mice within 6–10 days after birth, something that is never observed in humans.

The analysis of *nemo* knockout mice has provided valuable insights into the molecular and cellular events that may participate in IP dermatosis. It has been proposed that, upon an unknown kind of stress, necrosis of some *NEMO* (–) keratinocytes may happen after birth, triggering a local inflammation response and the secretion of cytokines such as TNF-α [14]. As a consequence, *NEMO* (–) cells would be extensively destroyed by TNF-α-induced apoptosis. According to this scenario, if elimination of all *nemo* (–) cells is not complete at this stage, a second wave of elimination may occur later. Such recurrence has indeed been observed in rare cases of IP, as explained above.

The mosaic state of *nemo* knockout female mice, as in IP patients, nevertheless makes a thorough analysis of IP dermatosis very difficult. As an alternative model, mice with selective *ikk-2* ablation in the epidermis have been produced [25]. Since NEMO and IKK-2 are two components of the same complex, their dysfunctions are likely to produce similar outcomes.

In *ikk-2* skin knockout mice, epidermis development proceeds normally till birth. At P4–P5 their skin starts becoming hard and inflexible and by day P7–P8 a widespread scaling is observed that precedes death. At P7, mice exhibit a thickened epidermis, and this hyperproliferation is accompanied with cell infiltration into the dermis and severe inflammation [25]. Several inflammatory cytokines, such as interleukin (IL)-1 and TNF-α, and chemokines accumulate in the epidermis or the dermis during progression of the disease. IL-1 is the first to be detected in the epidermis, before the onset of the pathology, preceding TNF-α accumulation in the dermis. The role of TNF-α in the development of the pathology appears essential since crossing *ikk-2* skin knockout mice with *tnf-r1* knockout mice completely suppresses the skin manifestations. Importantly, purified keratinocytes do not exhibit hyperproliferation on their own, indicating that the defect is not cell-autonomous. Thus, *ikk-2* skin knockout mice demonstrate that hyperproliferation is rather a secondary event resulting from inflammation.

In contrast to *nemo* knockout, apoptosis of keratinocytes in *ikk-2* skin knockout does not contribute substantially to the pathology whereas, as already stated, lesion clearance of IP patients results directly from *NEMO* (–) cell apoptosis.

Although identity of the signal(s) triggering the whole IP dermatosis process still remains unclear, the data summarized above suggest a plausible sequence of events and identify participants that orchestrate them. Among them, TNF-α appears to play a key role in both the onset of inflammation and its resolution through clearance of *NEMO*-mutated cells by apoptosis.

Anhidrotic ectodermal dysplasia with immunodeficiency (EDA-ID)

As indicated above, complete loss of NF-κB activation is lethal for males during embryogenesis, but females can survive because of their heterozygous status and the possibility of lyonization. For years, a rare and complex syndrome associating anhidrotic ectodermal dysplasia and immunodeficiency [EDA-ID (OMIM # 300291)] had been described, but its genetic cause was unknown [26–29]. Interestingly, all the reported cases were male, suggesting an X-linked mode of inheritance. This characteristic, together with the association with a perturbed immune response and some similarities with IP led to the analysis of the *NEMO* gene in several EDA-ID patients. Most of them indeed carry mutations in *NEMO*, but instead of leading to large truncations of the NEMO molecule as observed in IP, the mutations are missense mutations or small deletions only affecting the zinc finger located at the very C-terminus of the molecule [30–33] (fig. 2). Interestingly, all these mutations lead to reduced but not abolished NF-κB activation, explaining why affected male patients survive. Moreover, their single X chromosome carries the mutated gene, directly allowing observation of the physiological consequences of NF-κB dysfunction in humans.

The immunodeficiency affecting male patients with EDA-ID is characterized by unusually severe life-threatening and recurrent bacterial infections of lower respiratory tract, skin, soft tissues, bones and gastrointestinal tract, as well as meningitis and septicemia in early childhood. The causative pathogens are most often Gram-positive bacteria (*Streptococcus pneumoniae* and *Staphylococcus aureus*), followed by Gram-negative bacteria (*Pseudomonas* spp. and *Haemophilus influenzae*) and mycobacteria.

Most EDA-ID patients have hypogammaglobulinaemia with low serum immunoglobulin (Ig) G levels, while the levels of other immunoglobulin isotypes (IgA, IgM and IgE) can vary. A number of EDA-ID patients have been described with elevated serum IgM levels (the so-called hyper-IgM phenotype). In B cells, CD40 activates NF-κB through both NEMO-dependent and independent pathways. In some EDA-ID patients, B cells have an impaired ability to switch in response to CD40 ligand (CD40L). In others, the immunoglobulin switch is normal but the proliferation and activation is deficient, also resulting in a

hyper-IgM-like phenotype. A more consistent feature of EDA-ID pathology is impaired antibody response to polysaccharide antigens. In contrast to these B-cell anomalies, patients with EDA–ID have normal T-cell proliferation to mitogens and antigens. Recently, impaired NK activity has also been reported in several patients [34].

The high sensitivity of EDA-ID patients to infection result from an impaired cellular response of peripheral blood lymphocytes to LPS, IL-1β, IL-18, TNF-α and CD40L. LPS, a component of Gram – cell wall, is normally detected by TLR4, a member of the Toll-like receptor (TLR) family that triggers the innate immune response through NEMO-dependent NF-κB activation [35]. Other NF-κB-dependent pathways activated upon exposure to pathogens and involving other members of the TLR family are likely to be defective as well.

If immunodeficiency is not a surprising consequence of disturbed NF-κB activation, understanding its association with anhidrotic ectodermal dysplasia in EDA-ID is less straightforward. Anhidrotic ectodermal dysplasia (EDA) is a well-known pathology in which patients have no sweat glands, sparse scalp hair and rare conical teeth [36]. Three distinct loci, both in humans and mice, have been shown to be responsible for EDA. The first one, located on the X chromosome, codes for a member of the TNF family, ectodysplasin/EDA-A1 [37], a membrane-associated protein produced in cell types and tissues of ectodermal origin, such as keratinocytes, hair follicles and sweat glands [38]. Ectodysplasin is a type II transmembrane protein [39, 40] that is released as a trimer in the extracellular compartment after furin-dependent cleavage [41–43].

The second locus responsible for EDA is located on chromosome 2 and accounts for both autosomal recessive and dominant types of the disease [44, 45]. It encodes a death domain-containing member of the TNFR family, EDAR, and is a specific receptor for ectodysplasin [46–49]. Its expression is restricted to placodes, thickenings of epithelia where epidermal appendages begin to form [44].

The third locus whose disruption leads to EDA has been identified only recently in *crinkled* mice and subsequently found mutated in a human family [50, 51]. It is located on chromosome 1 in humans and 13 in mouse and encodes a death domain-containing adaptor protein EDARADD/CR, which binds EDAR through a homotypic death domain interaction. EDARADD is also able to interact with the adaptor molecule TRAF2.

Since members of the TNF/TNFR families are often connected to NF-κB signaling, the EDA syndrome caused by NF-κB dysfunction in EDA-ID can be deduced from what is stated above. Additional biochemical studies have confirmed that ectodysplasin/EDAR interaction indeed results in NF-κB activation [31, 46, 48]. Therefore, mutations in three genes that encode members of a signaling cascade that leads to NF-κB activation result in anhidrotic ectodermal dysplasia (fig. 3). Although the details of the ectodysplasin/EDAR signaling pathway are not fully characterized, it is clear from analyzing the mouse models of EDAR pathology that this pathway is involved very early during development of hair follicle morphogenesis [44], assigning a previously unrecognized role for NF-κB in this process.

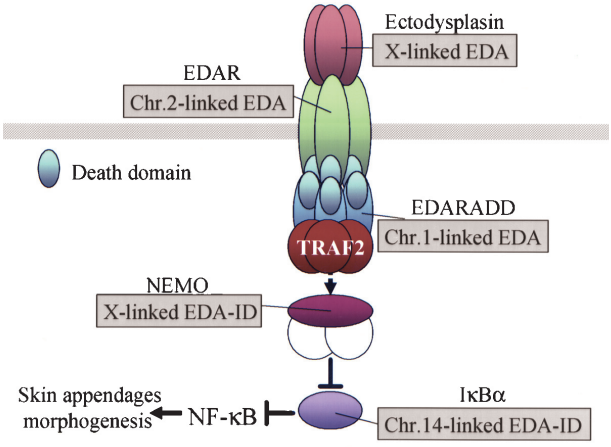

Figure 3. Ectodysplasin/EDAR signaling pathway. Molecules that are mutated in EDA and EDA-ID pathologies are indicated, together with their associated mode of genetic transmission (grey boxes).

In conclusion, both symptoms exhibited by EDA-ID patients can be reconciled with a defect in NF-κB activation: the immunological defect is due to the impairment of a series of important signaling pathways essential for the innate immune response, while the EDA symptom is specifically caused by an impairment of the downstream events of the ectodysplasin/EDAR/EDARADD signaling cascade.

EDA-ID with osteopetrosis and lymphoedema

Two EDA-ID patients exhibiting two additional defects, osteopetrosis and lymphoedema (OL-EDA-ID), have been described [21, 31, 52]. Osteopetrosis, which results from defective bone resorption by osteoclasts, can be easily tracked down to NF-κB, since it has been shown that p50/p52 knockout mice exhibit osteopetrosis [53, 54]. Moreover, the RANK pathway that plays an essential role in osteoclast function is itself NF-κB dependent [55]. Primary lymphoedema, a dysfunction specifically affecting lymphatic vessels, is still poorly understood at the genetic and biochemical level. Nevertheless, the gene causing familial lymphoedema, *V-EGFR-3*, has been identified, and overexpression of its product results in NF-κB activation [56].

Remarkably, the two reported OL-EDA-ID patients were found to carry the same genetic defect: replacement of

the *NEMO* stop codon with tryptophan, leading to the addition of 27 irrelevant amino acids at the C-terminus of the molecule [21, 31]. This seemingly benign mutation actually turned out to be very severe since it strongly destabilizes the NEMO molecule, leading to almost undetectable levels of this protein.

Other NEMO-related diseases

Two male patients suffering from immunodeficiency, without any associated sign of EDA, were very recently identified as mutated in the *NEMO* gene [57, 58]. The first one exhibited infections during childhood and subsequently developed an atypical mycobacteria infection. Several impaired immunologic functions were detected, among them reduced CD40-induced cell proliferation and variable TLR-induced TNF-α production. These defects were linked to a splice site mutation affecting exon 9 of *NEMO* with variable penetrance [57]. The second one experienced multiple infections (*Mycobacterium avium*, *H. influenzae*, *S. pneumoniae*) leading to osteomyelitis, dermatitis and bronchiectasis. He presented with an hyper-IgM phenotype and low interferon γ (IFN-γ) synthesis by peripheral blood mononuclear cells (PBMCs). The *NEMO* mutation identified in this case may result in synthesis of a protein lacking the first 37 amino acids [58].

Finally, a missense *NEMO* mutation (R254G) has been reported in a patient exhibiting a quite unorthodox phenotype, mostly characterized by idiopathic CD4 lymphopenia and chronic disseminated *M. avium* infection involving the lung, lymph nodes and bone marrow [59].

Genotype/phenotype correlation in NEMO-related diseases

Trying to establish genotype/phenotype correlations in NEMO-related diseases remains a major challenge but is worth the effort since it may provide important clues about how NF-κB acts in vivo. Unfortunately, the relatively unified picture of *NEMO* mutations leading to either IP or EDA-ID depending on the level of residual NF-κB activity, as presented above, has recently been questioned by the discovery of new mutations that are associated with pathologies exhibiting a severe phenotype despite a discrete genetic defect or presenting only partial signs of either condition.

Concerning the mutations that result in NEMO truncation (nonsense or frameshift mutations, IP rearrangement), the situation appears quite clear-cut. Deletions only affecting the NEMO zinc finger exhibit a residual NF-κB activation and are linked to EDA-ID whereas large deletions are associated with abolished NF-κB activation and cause IP (fig. 2). Remarkably, deletions extending into the Pro-rich domain of NEMO (around aa 384–391) generate EDA-ID when no (E391X nonsense mutations) or only few (frameshift mutations) irrelevant amino acids are added at the C-terminus of the molecule. In contrast, IP is generated when NEMO is further deleted (Q384X nonsense mutation) or when frameshifting appends a large amino acid extension. The molecular basis underlying the genetic switch from EDA-ID to IP that results from mutations affecting the aa 384–391 sequence of NEMO remains to be defined.

Things are less simple concerning the missense mutations. Indeed, several missense mutations of *NEMO* have been found associated with IP and sometimes with very severe forms of the disease. This is the case of mutation ΔK90, which has been identified in patients exhibiting severe neurological defects in addition to the classic signs of IP [24]. Interestingly, this mutation appears to specifically affect NEMO binding to IKK2 but not to IKK1. Another missense mutation located in the NEMO zinc finger has been identified in an IP patient [21], but it remains to be understood how it can generate the pathology. On the EDA-ID side, it is worth noting that point mutations causing this pathology are disseminated all along the NEMO molecule (fig. 2). Do they cause protein misfolding and destabilization or act more specifically? In the cases of A288G, D311N and zinc-finger mutations specific functions have been associated with domains affected. Both A288G and D311N mutations are located within the CC2/LZ domain that participates in NEMO oligomerization [60], and the zinc-finger mutations are supposed to affect the folding of a domain required for TNF-induced IKK activation [61].

The very complex genotype/phenotype relationships described above are not necessarily surprizing, given what we know about the NF-κB signaling pathway and the NEMO protein. Indeed, NF-κB is a ubiquitous transcription factor which participates in multiple signaling pathways, and NEMO appears to provide interfaces for most effector molecules used by these pathways. Accordingly, missense or discrete mutations may affect specific pathways and lead to various outcomes. Importantly, NEMO synthesis is also X-linked, and this directly impacts upon IP pathology. Thus, depending on the site where a mutation is expressed, at which time and what its impact is on a defined signaling pathway, elimination of mutated cells may occur or not in female patients, with benefit or trouble. This may be the case, for instance, for recently published *NEMO* mutations [24]. Their mild effect on cell homeostasis early during development may help the mutated cells to survive and accumulate later on, with deleterious consequences. In contrast, a selection against *NEMO* mutations at early stages may relieve the organism of mutated cells and result in less severe signs of IP.

IκBα-related disease

EDA-ID (autosomal-dominant form)

Two male patients exhibiting a severe form of EDA-ID but no mutation in *NEMO* have been identified [62,63]. Besides the manifestations shared with NEMO-related EDA-ID, they also show a specific T lymphocyte dysfunction. In vitro, T cell proliferation in response to mitogens or to recall antigens is strongly reduced, and no memory T cells are generated in vivo, despite lymphocytosis. Such a phenotype has not been observed in patients suffering from X-linked EDA-ID.

Based on the overall similarity of this disease with X-linked EDA-ID, an impaired NF-κB activation process was likely. Interestingly, patient-derived fibroblasts exhibited normal IKK activation, but IκBα degradation was specifically abolished and NF-κB activation appeared reduced. Upon sequencing of the *IKBA* gene, mutation at a phospho-acceptor site, Ser32, was identified in both cases. This residue, together with Ser36, is a target for IKK to induce IκBα degradation, explaining defective NF-κB activation. Since other IκBs are still functional, the NF-κB species under their control remain fully functional, and this residual activation explains the survival of male patients.

CYLD-related diseases

Familial cylindromatosis and multiple familial trichoepithelioma

Familial cylindromatosis/Spiegler-Brooke syndrome (OMIM #132700) is an autosomal dominant disease characterized by benign tumours (cylindromas) appearing during adulthood and exclusively derived from skin appendages such as eccrine and apocrine sweat glands. The gene causing cylindromatosis, *CYLD*, has been recently identified [64]. Based on its associated pathology, *CYLD* appears to code for a tissue-specific tumour supressor.

Cylindromas are supposed to develop from a stem cell compartment, the folliculosebaceous-apocrine unit that gives rise to various skin adnexes. Interestingly, inside the same family of cylindromatosis patients some members can also exhibit hair follicle tumors (trichoepitheliomas) intermingled with cylindromas [65]. Since multiple familial trichoepithelioma (MFT, OMIM #601606) is itself a well-recognized genetic defect, sequencing of the *CYLD* gene has been carried out in this disease. *CYLD* mutations have been identified in several distinct MFT families [66–68]. It therefore appears that both cylindromatosis and MFT share an identical genetic basis. From these observations it can be predicted that other less well known pathologies characterized by various adnexal neoplasms may also be caused by *CYLD* mutations.

Using two-hybrid screenings in yeast and NEMO as a bait, CYLD has been isolated, indicating its functional relationship to the NF-κB activation pathway [69, 70]. Besides binding to NEMO, CYLD has also been shown to interact with TRAF2 [69], an adaptor molecule of the TNF-α signaling pathway and TRIP [71]. Importantly, CYLD has also been recovered through another experimental approach, a whole-scale search aimed at identifying members of the deubiquitinase family controling NF-κB activation by TNF-α [72].

Upon overexpression CYLD has been shown to negatively regulate NF-κB activation induced by TNF-α, IL-1-β or CD40, and its deubiquitinase activity is required for this function [69–71]. Interestingly, it was been recently proposed that IKK activation may involve a ubiquitination event possibly targeting TRAFs, TAB2/3 (regulatory subunits of TAK1 kinase, a component of TNF-α and IL1/TLR signaling pathways) or NEMO [73–75]. Since polyubiquitin chains that are formed exclusively use Lysine 63 of Ubiquitin instead of Lysine 48 to build up chains, this ubiquitination does not trigger degradation. CYLD is likely to act at this level since it exhibits a restricted deubiquitinase specificity towards K63 chains.

Most mutations found in familial cylindromatosis or familial multiple trichoepithelioma are C-terminal deletions that affect the catalytic domain of CYLD. CYLD molecules exhibiting this class of mutation appear ineffective in negatively regulating NF-κB activation, reinforcing the notion that cylindromatosis is directly linked to perturbed NF-κB signaling. To explain how this defect may relate to the disease, it has been proposed that excessive protection against apoptosis resulting from uncontroled upregulation of NF-κB participates in the genesis of cylindromas. Attempts at blocking NF-κB activation and enhanced apoptosis resistance caused by CYLD dysfunction have been reported [72]. Nevertheless, it remains uncertain whether the use of NF-κB inhibitors will improve the condition of cylindromatosis patients since CYLD may also negatively regulate the JNK pathway, another important cancer-related signaling pathway [76].

Conclusion

Although NF-κB-related diseases were first identified only 5 years ago, much useful information has been gathered from the analysis of IP, EDA-ID and cylindromatosis pathologies. In particular, the formal proof that NF-κB plays a critical role in skin development and homeostasis derives directly from these studies. IP analysis has revealed that impaired NF-κB in the epidermis can trigger a complex dermatosis that combines inflammation, keratinocyte hyperproliferation and apoptosis. Defining this dermatosis in more detail may help understand the pathogenesis of other conditions showing similar signs of skin

inflammation and dysregulated keratinocyte proliferation. Moreover, the EDA syndrome of EDA-ID patients has for the first time allowed identification of the essential participation of NF-κB in the pathway that specifically controls morphogenesis of skin appendages. This relationship has been independently confirmed by uncovering a link between deubiquitinase CYLD, the protein associated with cylindromatosis, and NF-κB. Finally the crucial participation of NF-κB in various pathways controlling innate and acquired immune response has been confirmed in humans through the identification of X-linked and autosomal-linked EDA-ID pathologies, respectively. Considering the wide range of dysfunctions generated by mutations of a single component of the NF-κB pathway, NEMO, it can be predicted that mutations affecting others molecules of the same pathway will be found in the future associated with genetic diseases showing discrete or general perturbations of the skin and the haematopoietic compartment.

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