

Recent advances in understanding of the molecular basis of anhidrotic ectodermal dysplasia: discovery of a ligand, ectodysplasin A and its two receptors

Sławomir A. WIŚNIEWSKI, Agnieszka KOBIELAK, Wiesław H. TRZECIAK, Krzysztof KOBIELAK

Department of Biochemistry and Molecular Biology, K. Marcinkowski University of Medical Sciences, Poznań, Poland

Abstract. Recent developments of the investigations on the molecular basis of anhidrotic ectodermal dysplasia are reviewed. Identification of the major product of the *EDA* gene (ectodysplasin A), a protein belonging to a group of TNF ligands, and molecular cloning of the cDNA, encoding its receptor (EDAR), a member of the TNF receptor family, are presented. The role of an alternative EDA receptor, localised on the X chromosome (XEDAR) in the developmental control of the differentiation of skin appendages, is discussed. Recent findings have elucidated the cause of the autosomal forms of EDA, both dominant and recessive, and indicated an important role of a signal transduction pathway involving a protein product of the *NEMO* gene and the transcription factor NF κ B in the differentiation of skin appendages.

Key words: anhidrotic ectodermal dysplasia, genes, TNF-family, ligand, receptor.

Introduction

Anhidrotic (hypohidrotic) ectodermal dysplasia (EDA) (MIM #305100; MCKUSICK 1998), known also as Christ-Siemens-Touraine syndrome, is the most common form of approximately 150 related disorders, and results from impaired development of skin appendages during embryogenesis. The syndrome is characterised by the absence or deficient function of at least two derivatives of

Received: December 2, 2001. Accepted: December 8, 2001.

Correspondence: W.H. TRZECIAK, Department of Biochemistry and Molecular Biology, ul. Świecickiego 6, 60-781 Poznań, Poland, e-mail: trzeciak@am.poznan.pl

the ectoderm: teeth, hair, sweat glands or nails. Various clinical forms of the disease are due to different modes of inheritance. The most common variant involves an X-linked recessive inheritance, with partial manifestation in females. The affected individuals exhibit symptoms of *anodontia* or *oligodontia* with conical shape of teeth, *hyperthermia* caused by severe deficiency of sweat glands and *hypotrichosis* – sparse hair. Many individuals present a characteristic facial appearance including a prominent forehead, “saddle nose” and unusually thick lips. In some patients immune deficiency is also observed (MCKUSICK 1998).

Although in most of the cases an X-linked inheritance can be demonstrated, the disease may also be transmitted as an autosomal dominant or recessive trait (JORGENSEN et al. 1987, HO et al. 1998, MUNOZ et al. 1997, BAALA et al. 1999, HEADON, OVERBEEK 1999, MONREAL et al. 1999).

Since the publishing of our previous reviews ((KOBIELAK et al. 1997, 1999a) a considerable progress has been made towards understanding the molecular events underlying differentiation of skin appendages and consequently elucidating the cause of autosomal forms of EDA, both dominant and recessive. The structure of the protein product of the *EDA* gene has been resolved (MONREAL et al. 1998) and it has been found that in addition to isoform 1 (KERE et al. 1996), there are two variants of isoform 2 (named ectodysplasin A1 and A2). These variants belong to the family of tumour necrosis factor (TNF) ligands (EZER et al. 1999). The discovery of their receptors EDAR and XEDAR (MONREAL et al 1999, YAN et al. 2000), members of the TNF receptor family, has directed research towards better understanding of signal transduction pathways involved in the developmental control of differentiation of tooth buds, hair follicles and eccrine sweat glands.

The purpose of this review is to present and discuss recent progress in research on the molecular basis of anhidrotic ectodermal dysplasia.

Identification of the protein product of the *EDA* gene

Investigations on the molecular basis of anhidrotic ectodermal dysplasia were prompted by the studies conducted in Tabby mice. Tabby mice exhibit symptoms of ectodermal dysplasia due to defects in the development of tooth buds, hair follicles, sweat glands and tail, caused by mutations localised on the X-chromosome (SOFAER 1969).

On the basis of the animal phenotype FERGUSON et al. (1997) identified and cloned the *tabby* gene, a murine equivalent of the human *EDA* gene cloned one year earlier by KERE et al. (1996). The primary transcript of the *EDA* gene originally described (designated isoform I), contained two exons separated by a 2kb intron, and encoded a 135-amino-acid transmembrane protein. However, the cloned *tabby* gene encoded additional 246 amino acids of the protein product, including a short collagenous domain (SRIVASTAVA et al. 1997). This discovery facilitated the identification of isoform II of the *EDA* gene product. The predicted transmembrane protein comprised 391 amino acid residues, including a collagen-like motif containing 19 Gly-X-Y repeats and a sequence conserved in

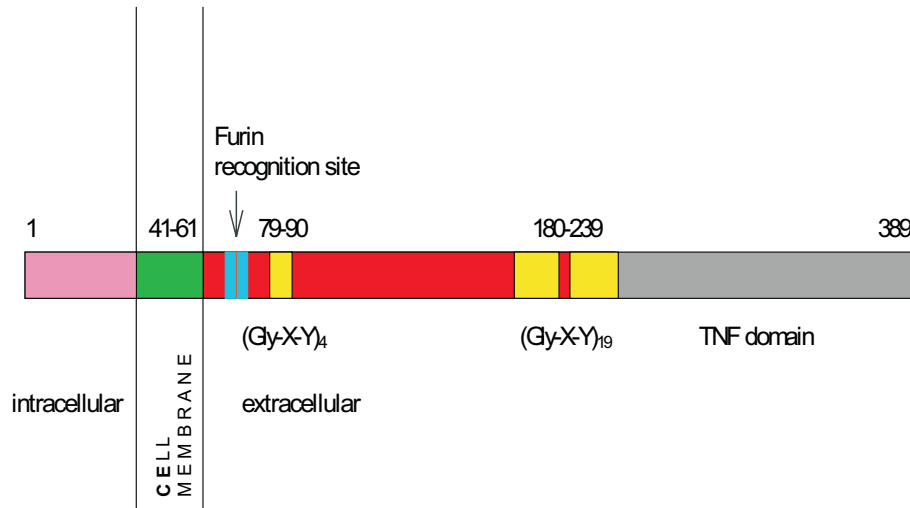


Figure. 1. Schematic representation of Ectodysplasin structure

the TNF ligand family, both localised in the extracellular domain (Figure 1). MONREAL et al. (1998) established the genomic structure of the *EDA* gene and the complete sequences of the 7 new exons were determined in 18 males with an X-linked hypohidrotic ectodermal dysplasia. The results suggested that *EDA* isoform II plays a critical role in the morphogenesis of tooth, hair, and sweat

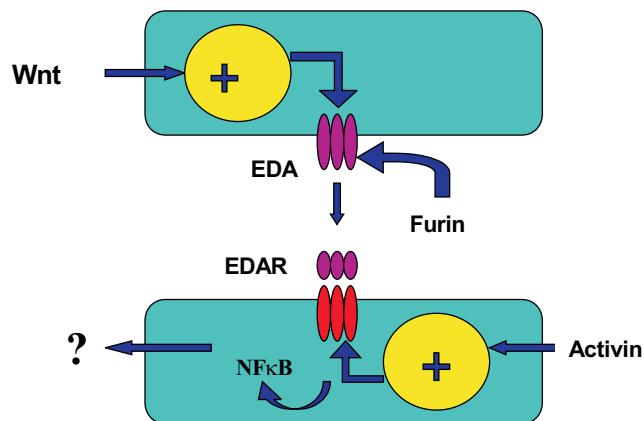


Figure. 2. Hypothetical signalling pathway involving Ectodysplasin A1 and its receptor EDAR. Ectodysplasin A1 trimer is cleaved by furin and as a soluble ligand can interact with EDAR.

glands, whereas the biological significance of isoform I was unclear. BAYES et al. (1998) reported three mutations that removed either two or four Gly-X-Y repeats, without interrupting the reading frame, thus suggesting a functional role for the collagenous domain. EZER et al. (1999) found that isoform II is a trimeric type II membrane protein that co-localises with cytoskeleton structures at the lateral and apical surfaces of the cells. The TNF-homology domain is similar to other members of the TNF family of ligands and is composed of ten antiparallel sheets linked by variable loops. The closest homologues of ectodysplasin A in the TNF family of ligands are BAFF/BlyS, APRIL and TWEAK (MOORE et al. 1999, SCHNEIDER et al. 1999, HAHNE et al. 1998, CHICHEPORTICHE et al. 1997), although none of them contains Gly-X-Y repeats. However, all four TNF ligands including ectodysplasin A comprise a consensus sequence, which constitutes a potential binding site for a protease called furin, involved in the proteolytic cleavage of the extracellular domain, thus making the ligands soluble (Figure 2). In fact, ectodysplasin A contains two overlapping furin-binding sites located be-

Table 1. Location of mutations in functional domains of *EDA* gene resulting in anhidrotic ectodermal dysplasia phenotype

Exons	Domain	Number of mutations	Reference
1	Intracellular (1-40)	4	KERE et al. 1996 BAYES et al. 1998 FERGUSON et al. 1998
1	Transmembrane (41-63)	4	KERE et al. 1996 HERTZ et al. 1998 MARTINEZ et al. 1999 YOTSUMOTO et al. 1998
1	Extracellular (64-132)	8	FERGUSON et al. 1998 KERE et al. 1996
3	Furin sub-domain (150-159)	21	BAYES et al. 1998 MONREAL et al. 1998 AOKI et al. 2000 KOBIELAK et al. 2001a
5 and 6	Collagen-like sub-domain (180-235)	26	BAYES et al. 1998 MONREAL et al. 1998 KOBIELAK et al. 2000b
7-9	TNF homology sub-domain (250-391)	26	BAYES et al. 1998 MONREAL et al. 1998 KOBIELAK et al. 2001a

tween the transmembrane domain and the stretch of Gly-X-Y repeats. Recently SCHNEIDER et al. (2001) identified 44 mutations (including 17 novel ones) in patients with anhidrotic ectodermal dysplasia. These mutations clustered in three functionally important domains of the protein: (1) TNF-homology domain, responsible for binding to the receptor; (2) collagen-like domain, indispensable for

trimerisation of the ligand; and (3) the protease cleavage site, which determines solubility of the ligand. Recently, we have described three patients with typical symptoms of ectodermal dysplasia and almost identical phenotype (KOBIELAK et al. 2001a). Nevertheless, each patient harboured a different type of mutation: a novel frameshift mutation which resulted in a truncated form of the ligand, a mutation that generated an additional glycosylation site in the extracellular domain, and a mutation localised in a conserved region with a high homology to the TNF ligands. In addition, several polymorphisms in the intronic sequences of the *EDA* gene have been reported, including two rare ones (KOBIELAK et al. 2001b). However, the significance of these polymorphisms for the function of the protein product of the *EDA* gene remains unknown. All mutations found so far in the *EDA* gene are clustered in the functional domain of the ligand (Table 1).

These findings taken together strongly suggest that ectodysplasin A is a novel member of the TNF ligand family, involved in the early epithelial-mesenchymal interactions that regulate the formation of skin appendages, and its mutations located in the functional regions of the extracellular domain underlay the phenotype of anhidrotic ectodermal dysplasia.

Cloning of the *DL* gene

The discovery of the *dl* gene could be traced back to SOFAER (1969) who noticed that mutations localised in two different chromosomal loci in mice, designated as downless (*dl*) and crinkled (*cr*), resulted in the same abnormalities of teeth and hair as those observed in Tabby mice. Subsequently HEADON and OVERBEEK (1999) identified the murine *dl* gene, which encodes a novel member of the TNF receptor family. The phenotype of the mutant and the expression pattern of the *dl* gene suggest that this gene is responsible for the differentiation of tooth buds and hair follicles. Its ligand is likely to be a product of the *tabby* gene, since this gene encodes a protein highly homologous to the TNF ligands. Northern blot analysis of mRNA extracted from mouse skin on embryonic day 17.5 revealed a 3.8-kb transcript of the *dl* gene. On embryonic day 13, prior to the induction of hair follicle growth, the *dl* gene was expressed throughout the basal layer of the epidermis. On embryonic day 15, its expression was up-regulated in the cells initiating and undergoing follicular morphogenesis, and down-regulated in the surrounding cells. On embryonic day 17, the *dl* gene was expressed in elongating primary follicles and secondary follicles, but was no longer detected in the inter-follicular epidermis. MONREAL et al. (1999) isolated and characterised a human homologue of the *dl* gene. This gene, designated *DL* or *EDAR*, encodes a membrane protein comprising 448 amino acids, 91% identical with the protein product of the murine *dl* gene. They demonstrated that the *DL* gene is located on chromosome 2q11-q13. The structural analysis of the gene showed the presence of 12 exons and their flanking intronic sequences. Protein modelling revealed partial match with the cysteine-rich region of the ligand-binding domain of the TNF α receptor. Moreover, sequence similarity to the *death domain* of the TNF family of receptors

was identified. This domain resides in the carboxy-terminus of the protein and allows trimerisation and interaction with downstream signal-transducing proteins. Sequence homology of the two domains to the respective domains of receptors belonging to the TNF family, suggests that the protein product of the *DL* gene functions as a type I transmembrane protein and is structurally related to the TNF receptors. In search of mutations in the *DL* gene in three families displaying the recessive inheritance, and in two with the dominant inheritance, MONREAL et al. (1999) identified seven sequence variants; two of them were also detected in the general population. The mutations found in the autosomal recessive form of anhidrotic ectodermal dysplasia, however, did not map to the candidate-gene locus in all families, suggesting the existence of at least one additional human locus.

The results of those investigations imply that the protein product of the *DL* gene is a receptor capable of binding ectodysplasin A and mutations in the *DL* gene are responsible for the clinical symptoms of the autosomal forms of ectodermal dysplasia.

Discovery of the *XEDAR* gene

YAN et al. (2000) found that isoform II of the *EDA* gene product (designated EDA-A1) specifically binds to DL (EDAR). They also discovered an alternative transcript of the *EDA* gene, encoding a protein that is identical to EDA-A1 except for the deletion of two adjacent amino acids, Glu308 and Val309. This variant, designated EDA-A2, exclusively binds to an X-linked ectodysplasin receptor (XEDAR). The deduced amino acid sequence of XEDAR is similar to that of DL (EDAR) and TROY (KOJIMA et al. 2000). The receptor contains three cysteine-rich repeats and a single transmembrane domain but lacks the N-terminal signal peptide. This protein belongs to type III transmembrane receptors with an extracellular N-terminal domain and an intracellular C-terminal domain. However, XEDAR failed to bind a number of ligands belonging to the TNF family. Expression of XEDAR caused activation of nuclear factor- κ B (NF- κ B). It has been demonstrated that the intracellular domain of XEDAR binds the TNF receptor-associated factor-6 (TRAF-6), the TRAF-6 binding site being formed by amino acids 252 to 260. Because the binding of TRAF-6 to XEDAR correlates with the activation of NF- κ B, supposedly TRAF-6 is the key adaptor molecule involved in XEDAR-mediated signal transduction. The expression of the *XEDAR* gene was barely detectable until embryonic days 16 and 17, when it was expressed in large amounts in maturing hair follicles. By postnatal day 1, its expression was confined to hair follicles. The EDA-A2, which binds to XEDAR, showed expression in the core of the developing hair follicle, whereas expression of EDA-A1, which binds to EDAR, was circumferential.

These results strongly suggest that XEDAR belongs to the TNF family of receptors and binds an isoform of ectodysplasin A, designated EDA-A1, thus constituting part of the novel signal transduction pathway involved in the differentiation of skin appendages.

Discovery of the *NEMO* gene

YAMAOKA et al. (1998) characterised a mutant cell line, 5R, unresponsive to NF κ B. Using a genetic complementation approach, they cloned a component of the NF κ B inhibitor (I κ B)-kinase- γ complex, termed *NEMO*, for NF κ B essential modulator. The 2.8kb *NEMO* cDNA encodes a 412-amino acid protein, rich in glutamic acid and glutamine residues. They concluded that the defective phenotype of 5R cells resulted from the absence of the NEMO protein. Two years later the sequence of the complete *NEMO* locus was established (JIN, JEANG 1999). The *NEMO* gene, 23kb in length, is composed of 10 exons and contains three alternative noncoding first exons (JIN, JEANG 1999, ROTHWARF et al. 1998, LI et al. 1999). By sequence alignment, JIN and JEANG (1999) mapped the *NEMO* gene to chromosome Xq28. Since I κ B-kinase- γ plays an important role in the function of B- and T-lymphocytes, ZONANA et al. (2000) hypothesised that the association of the defects in differentiation of skin appendages with the immune defect in the X-linked ectodermal dysplasia, might be due to a mutation in the *NEMO* gene. They studied affected members of four families with anhidrotic ectodermal dysplasia and immunodeficiency, in whom the disorder segregated as an X-linked recessive trait. In all affected individuals, they found mutations in exon 10 of the *NEMO* gene encoding the C-terminus of I κ B-kinase- γ . The role of mutations in the *NEMO* gene was further substantiated by DOFFINGER et al. (2001) who identified five kindreds with anhidrotic ectodermal dysplasia and immunodeficiency. In all the patients, the symptoms of ectodermal dysplasia were somewhat milder than in children with anhidrotic ectodermal dysplasia without concomitant immunodeficiency. They also demonstrated that the DL protein (EDAR) affects NF- κ B through the mediation of the NEMO protein, indicating that anhidrotic ectodermal dysplasia results from impaired NF κ B signalling.

These observations, taken together, implicated that NF κ B is an important component of signal transduction pathway involving the variant of ectodysplasin A (EDA-A1) as a ligand, and the protein product of the *DL* gene as a receptor.

Concluding remarks

The discovery of the two receptors capable of binding the variants of ectodysplasin A has prompted research towards elucidation of signal transduction pathways involved in the differentiation of skin appendages during embryogenesis. The participation of a transcription factor NF- κ B and a protein product of the *NEMO* gene in transducing signals, essential for formation of tooth buds, hair follicles and sweat glands, suggests that the process of apoptosis is involved in differentiation of these structures. It is still unknown, however, how the process is triggered. Cloning and functional characterisation of the *EDA* gene promoter (PENGUE et al. 1999) has opened the possibilities of studying the expres-

sion of this gene. Since a sequence element called HK-1 motif, capable of binding transcription factor LEF-1, was discovered in the regulatory region of the *EDA* gene promoter (PENGUE et al. 1999), and a mutation adjacent to the HK-1 motif was found in the patient with anhidrotic ectodermal dysplasia (KOBIELAK et al. 1998), we have recently cloned rat (KOBIELAK et al. 1999b) and human (KOBIELAK et al. 2001c) LEF-1 and expressed the cloned cDNA in *E. coli* in order to investigate the role of LEF-1 in the expression of the *EDA* gene. The results revealed that LEF-1, acting in a complex with β -catenin, negatively regulates expression of the *EDA* gene, suggesting involvement of the Wnt pathway in the differentiation of skin appendages (KOBIELAK et al. 2000a). However, further investigations are required to elucidate the molecular events underlying the effect of LEF-1 and other components of the Wnt pathway on the initiation of the expression of the *EDA* gene.

REFERENCES

- AOKI N., ITO K., TACHIBANA T., ITO M. (2000). A novel arginine \rightarrow serine mutation in *EDA1* in a Japanese family with X-linked anhidrotic ectodermal dysplasia. *J. Invest. Dermatol.* 115: 329-330.
- BAALA L., RABIA S.H., ZLOTOGORA J., KABBAJ K., CHFIOUL H., MUNNICH A., LYQNNET S., SEFANI A. (1999). Both recessive and dominant forms of anhidrotic/hypohidrotic ectodermal dysplasia map to chromosome 2q1 1-q13. *Am. J. Hum. Genet.* 64: 651-653.
- BAYES M., HARTUNG A.J., EZER S., PISPA J., THESLEFF I., SRJVASTAVA A.K., KERE J. (1998). The anhidrotic ectodermal dysplasia gene (*EDA*) undergoes alternative splicing and encodes ectodysplasin-A with deletion mutations in collagenous repeats. *Hum. Molec. Genet.* 7: 1661-1669.
- CHICHEPORTICHE Y., BOURDON P.R., XU H., HSU Y.M., SCOTT H., HESSION C., GARCIA I., BROWNING J.L. (1997). TWEAK, a new secreted ligand in the tumor necrosis factor family that weakly induces apoptosis. *J. Biol. Chem.* 272: 32401-32410.
- DOFFINGER R., SMAHI A., BESSIA C., GEISSMANN F., FEINBERG J., DURANDY A., BODEMER C., KENWRICK S., DUPUIS-GIROD S., BLANCHE S., WOOD P., RABIA S.H., HEADON D.J., OVERBEEK P.A., LE DEIST F., HOLLAND S.M., BELANI K., KUMARARATNE D.S., FISCHER A., SHAPIRO R., CONLEY M.E., REIMUND E., KALHOFF H., ABINUN M., MUNNICH A., ISRAEL A., COURTOIS G., CASANOVA J.L. (2001). X-linked anhidrotic ectodermal dysplasia with immunodeficiency is caused by impaired NF-kappaB signaling. *Nature Genet.* 27: 277-285.
- EZER S., BAYES M., ELOMAA O., SCHLESSINGER D., KERE J. (1999). Ectodysplasin is a collagenous trimeric type II membrane protein with a tumour necrosis factor-like domain and colocalizes with cytoskeletal structures at lateral and apical surfaces of cells. *Hum. Molec. Genet.* 8: 2079-2086.
- FERGUSON B.M., BROCKDORFF N., FORMSTONE E., NGYUEN T., KRONMILLER J.E., ZONANA J. (1997). Cloning of *Tabby*, the murine homologue of the human *EDA* gene: evidence for a membrane-associated protein with a short collagenous domain. *Hum. Molec. Genet.* 6: 1589-1594.

- FERGUSON B.M., THOMAS N.S. T., MIJNOZ F., MORGAN D., CLARKE A., ZONANA J. (1998). Scarcity of mutations detected in families with X linked hypohidrotic ectodermal dysplasia: diagnostic implications. *J. Med. Genet.* 35: 112-115.
- HEADON D.J., OVERBEEK P.A. (1999). Involvement of a novel Tnf receptor homologue in hair follicle induction. *Nature Genet.* 22: 370-4.
- HAHNE M., KATAOKA T., SCHROTER M., HOFMANN K., IRMLER M., BODMER J.L., SCHNEIDER P., BORNARD T., HOLLER N., FRENCH L.E., SORDAT B., RIMOLDI D., TSCHOPP J. (1998). APRIL, a new ligand of the tumor necrosis factor family, stimulates tumor cell growth. *J. Exp. Med.* 188: 1185-1190.
- HERTZ J.M., NORGAARD HANSEN K., JUNCKER I., KJELDSSEN M., GREGERSEN N. (1998). A novel missense mutation (402C→T) in exon 1 in the *EDA* gene in a family with X-linked hypohidrotic ectodermal dysplasia. *Clin. Genet.* 53: 205-209.
- HO L., WILLIAMS M.S., SPRITZ R.A. (1998). A gene for autosomal dominant hypohidrotic ectodermal dysplasia (*EDA3*) maps to chromosome 2q1 1-q13. *Am. J. Hum. Genet.* 62: 1102-1106.
- JIN D.Y., JEANG K.T. (1999). Isolation of full-length cDNA and chromosomal localization of human NF-kappaB modulator NEMO to Xq28. *J. Biomed. Sci.* 6: 115-120.
- JORGENSEN R.J., DOWBEN J.S., DOWBEN S.L. (1987). Autosomal dominant ectodermal dysplasia. *J. Craniofac. Genet. Dev. Biol.* 7: 403-412.
- KERE J., SRIVASTAVA A.K., MONTONEN O., ZONANA J., THOMAS N., FERGUSON B., MUNOZ F., MORGAN D., CLARKE A., BAYBAYAN P., CHEN E.Y., EZER S., SAARIALHO-KERE U., DE LA CHAPELLE A., SCHLESSINGER D. (1996). X-linked anhidrotic (hypohidrotic) ectodermal dysplasia is caused by mutation in a novel transmembrane protein. *Nature Genet.* 13: 409-416.
- KOBIELAK K., KOBIELAK A., LIMON J., TRZECIAK W.H. (1998). Mutation in the regulatory region of the *EDA* gene coincides with the symptoms of anhidrotic ectodermal dysplasia. *Acta Biochim. Polon.* 45: 245-250.
- KOBIELAK K., KOBIELAK A., TRZECIAK W.H. (1997). Is the recently discovered *EDA* gene associated with the symptoms of anhidrotic ectodermal dysplasia? *J. Appl. Genet.* 38: 343-357.
- KOBIELAK K., KOBIELAK A., TRZECIAK W.H. (1999a). Novel isoforms of transcript of the *EDA* gene confirm X-linked inheritance of anhidrotic ectodermal dysplasia. *J. Appl. Genet.* 40: 355-364.
- KOBIELAK K., KOBIELAK A., TRZECIAK W.H. (1999b). Cloning of the lymphoid enhancer binding factor-1 (Lef-1) cDNA from rat kidney: homology to the mouse sequence. *Acta Biochim. Polon.* 46: 885-888.
- KOBIELAK K., KOBIELAK A., TRZECIAK W.H. (2000a). The influence of the transcription factor LEF-1 on the expression of the *EDA* gene. 18th International Congress of Biochemistry, Birmingham, Abstract book 1991.
- KOBIELAK K., KOBIELAK A., ROSZKIEWICZ J., LIMON J., TRZECIAK W.H. (2000b). Recurrent deletion of the region encoding two (Gly-X-Y) repeats in patients with anhidrotic ectodermal dysplasia indicates important role for collagen-like domain of the *EDA* gene product-ectodysplasin A. *Ped. Path. Mol. Med.* 19: 425-432.

- KOBIELAK K., KOBIELAK A., ROSZKIEWICZ J., WIERZBA J., LIMON J., TRZECIAK W.H. (2001a). Mutations in the *EDA* gene in three unrelated families reveal no apparent correlation between phenotype and genotype in the patients with an X-linked anhidrotic dysplasia. *Am. J. Med. Genet.* 100: 191-197.
- KOBIELAK A., KOBIELAK K., WIŚNIEWSKI S.A., MIDRO A.T., TRZECIAK W.H. (2001b). Sequence polymorphisms of the *EDA* and the *DL* genes in the patients with an X-linked and autosomal forms of anhidrotic ectodermal dysplasia. *Folia Histochem. Cytobiol.* 39: 113-114.
- KOBIELAK A., KOBIELAK K., TRZECIAK W.H. (2001c). A novel isoform of human lymphoid enhancer-binding factor-1 (*LEF-1*) gene transcript encodes a protein devoid of HMG domain and nuclear localization signal. *Acta Biochim. Polon.* 48: 221-226.
- KOJIMA T., MORIKAWA Y., COPELAND N.G., GILBERT D.J., JENKINS N.A., SENBA E., KITAMURA T. (2000). *TROY*, a newly identified member of the tumor necrosis factor receptor superfamily, exhibits a homology with *Edar* and is expressed in embryonic skin and hair follicles. *J. Biol. Chem.* 275: 20742-20747.
- LI Z.W., CHU W., HU Y., DELHASE M., DEERINCK T., ELLISMANN M., JOHNSON R., KARIN M. (1999). The IKK β subunit of I κ B kinase (IKK) is essential for nuclear factor κ B activation and prevention of apoptosis. *J. Exp. Med.* 189: 1839-1845.
- MARTINEZ F., MILLAN J.M., ORELLANA C., PRIETO F. (1999). X-linked anhidrotic (hypohidrotic) ectodermal dysplasia caused by a novel mutation in *EDA1* gene: 406T \rightarrow G (Leu55Arg). *J. Invest. Dermatol.* 113: 285-286.
- MCKUSICK V.A. (1998). Mendelian inheritance in man. 12th edn. Johns Hopkins University Press, Baltimore.
- MONREAL A.W., ZONANA J., FERGUSON B. (1998). Identification of a new splice form of the *EDA1* gene permits detection of nearly all X-linked hypohidrotic ectodermal dysplasia mutations. *Am. J. Hum. Genet.* 63: 380-389.
- MONREAL A.W., FERGUSON B., HEADON D.J., STREET S.L., OVERBEEK P.A. (1999). Mutations in the human homologue of mouse *dl* cause autosomal recessive and dominant hypohidrotic ectodermal dysplasia. *Nature Genet.* 22: 366-369.
- MOORE P.A., BELVEDERE O., ORR A., PIERI K., LAFLEUR D.W., FENG P., SOPPET D., CHARTERS M., GENTZ R., PARMELEE D., LI Y., GALPERINA O., GIRI J., ROSCHKE V., NARDELLI B., CARRELL J., SOSNOVTSEVA S., GREENFIELD W., RUBEN S.M., OLSEN H.S., FIKES J., HILBERT D.M. (1999). BLYS: member of the tumor necrosis factor family and B lymphocyte stimulator. *Science* 285: 260-263.
- MUNOZ F., LESTRINGANT G., SYBERT V., FRYDMAN M., ALSWAJNI A., FROSSARD P.M., JORGENSEN R., ZONANA J. (1997). Definitive evidence for an autosomal recessive form of hypohidrotic ectodermal dysplasia clinically indistinguishable from the more common X-linked disorder. *Am. J. Hum. Genet.* 61: 94-100.
- PENGUE G., SRIVASTAVA A.K., KERE J., SCHLESSINGER D., DURMOWICZ M.C. (1999). Functional characterization of the promoter of the X-linked ectodermal dysplasia gene. *J. Biol. Chem.* 274: 26477-26484.
- ROTHWART D.M., ZANDI E., NATOLI G., KARIN M. (1998). IKK-gamma is an essential regulatory subunit of the I κ B kinase complex. *Nature* 395: 297-300.
- SCHNEIDER P., MACKAY F., STEINER V., HOFMANN K., BODMER J.L., HOLLER N., AMBROSE C., LAWTON P., BIXLER S., ACHA-ORBEA H., VALMORI D., ROMERO P.,

- WERNER-FAVRE C., ZUBLER R.H., BROWNING J.L., TSCHOPP J. (1999). BAFF, a novel ligand of the tumor necrosis factor family, stimulates B cell growth. *J. Exp. Med.* 189: 1747-1756.
- SCHNEIDER P., STREET S.L., GAIDE O., HERTIG S., TARDIVEL A., TSCHOPP J., RUNKEL L., ALEVIZOPOULOS K., FERGUSON B.M., ZONANA J.(2001). Mutations leading to X-linked hypohidrotic ectodermal dysplasia affect three major functional domains in the tumor necrosis factor family member ectodysplasin-A. *J. Biol. Chem.* 276: 18819-18827.
- SOFAER J.A. (1969). Aspects of the tabby-crinkled-downless syndrome. *J. Embryol. Exp. Morph.* 22: 181-205.
- SRIVASTAVA A.K., PISPA J., HARTIJNG A.J., DU Y., EZER S., JENXS T., SHIMADA T., PEKKANEN M., MIKKOLA M.L., KO M.S.H., THIESLEFF I., KERE J., SCHLESSINGER D. (1997). The Tabby phenotype is caused by mutation in a mouse homologue of the EDA gene that reveals novel mouse and human exons and encodes a protein (ectodysplasin A) with collagenous domains. *Proc. Natl. Acad. Sci. USA* 4: 13069-13074.
- YAMAOKA S., COURTOIS G., BESSIA C., WHITESIDE S.T., WEIL R., AGOU F., KIRK H.E., KAY R.J., ISRAEL A. (1998). Complementation cloning of NEMO, a component of the I κ B kinase complex essential for NF- κ B activation. *Cell* 93: 1231-1240.
- YAN M., WANG L.C., HYMOWITZ S.G., SCHILBACH S., LEE J., GODDARD A., DE VOS A.M., GAO W.Q., DIXIT V.M. (2000). Two-amino acid molecular switch in an epithelial morphogen that regulates binding to two distinct receptors. *Science* 290: 523-527.
- YOTSUMOTO S., FUKUMARU S., MATSUSHITA S., OKU T. KOBAYASHI K., SAHEKI T., KANZAKI T. (1998). A novel point mutation of the EDA gene in a Japanese family with anhidrotic ectodermal dysplasia. *J. Invest. Dermatol.* 111: 1246-1247.
- ZONANA J., ELDER M.E., SCHNEIDER L.C., ORLOW S.J., MOSS C., GOLABI M., SHAPIRA S.K., FARNDON P.A., WARA D.W., EMMAL S.A., FERGUSON B.M. (2000). A novel X-linked disorder of immune deficiency and hypohidrotic ectodermal dysplasia is allelic to incontinentia pigmenti and due to mutations in IKK-gamma (NEMO). *Am. J. Hum. Genet.* 67: 1555-1562.