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A novel discoidin domain receptor 1 (*Ddr1*) transcript is expressed in postmeiotic germ cells of the rat testis depending on the major histocompatibility complex haplotype

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Abstract

The *Ddr1* gene encoding the discoidin domain receptor 1 (DDR1), a member of a small subfamily of receptor tyrosine kinases, is known to be involved in differentiation, proliferation, and cell adhesion. The extracellular discoidin domain is responsible for the binding of the ligand collagen. As the human homologue, the rat *Ddr1* gene consists of 17 exons and is located in the major histocompatibility complex, the RT1 complex in rats. A novel testis-specific *Ddr1* transcript of 3.5 kb is described here which is expressed specifically in the postmeiotic germ cells of the rat testis. The exons 1 to 5 are missing in this transcript and the putative protein would lack the discoidin domain and parts of the stalk region. The expression level of both, the full-length 4.3 kb and the novel 3.5 kb *Ddr1* transcript, is dependent on the RT1 haplotype. In the $RT1^{avl}$ haplotype, carried by DA and LEW.1AV1 rats, the 3.5 kb *Ddr1* transcript is completely missing. This might be explained by the lack of four nucleotides GGGC in the $RT1^{avl}$ haplotype, which appear to contribute to a SP1 binding site in intron 5 of the *Ddr1* gene in the presumed testis-specific alternative promoter region of the 3.5 kb *Ddr1* transcript. In addition, two novel exons in the 5′-untranslated region of the *Ddr1* gene were found that give rise to further alternative *Ddr1* transcripts. Interestingly, the 3.5 kb *Ddr1* transcript is not only expressed in a cell type-specific manner in postmeiotic germ cells but also controlled by the RT1 haplotype.

Keywords: Alternative splicing; RT1 haplotypes; Receptor tyrosine kinases; Spermatogenesis

1. Introduction

Receptor tyrosine kinases (RTKs) are involved in proliferation, differentiation, and migration of cells. Ligand binding induces receptor dimerization and autophosphorylation of specific tyrosine residues in the cytoplasmic part of the proteins. Discoidin domain receptor 1 (DDR1) represents a small subfamily of RTKs found predominantly in epithelial cells (Alves et al., 1995). DDR1 is encoded in the major histocompatibility complex (MHC) and encompasses 17 coding exons. The extracellular domain of DDR1 consists of a discoidin domain encoded by exons 1 to 3 and the stalk region corresponding to exons 4 to 8. The transmembrane domain is encoded by exon 9, the juxtamembrane region by exons 10 to 12 and the C-terminal

Abbreviations: cDNA, DNA complementary to RNA; d, day; DDR1, discoidin domain receptor 1; DMEM, Dulbecco's modified Eagles Medium; dNTP, deoxyribonucleoside triphosphate; EST, expressed sequence tag; kb, kilobases; MHC, major histocompatibility complex; min, minute; PCR, polymerase chain reaction; RACE, rapid amplification of cDNA ends; RNase, ribonuclease; RT-PCR, reverse transcriptase PCR; RTK, receptor tyrosine kinase; SDS, sodium dodecyl sulfate; SSC, 0.15 M NaCl/0.015 M Na_3·citrate pH 7.6; UTR, untranslated region.

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catalytic tyrosine kinase domain by exons 13 to 17. The ligand of the DDR1 receptor is collagen in its native triple-helical structure. The discoidin domain of DDR1 binds to collagen (Vogel et al., 1997). The analysis of *Ddr1*-deficient mice indicated that DDR1 influences cell morphogenesis, differentiation, proliferation, and adhesion (Vogel et al., 2001; Curat and Vogel, 2002). Furthermore, DDR1 has been reported to be overexpressed in certain carcinomas (Alves et al., 1995; Perez et al., 1996) and brain tumors (Weiner et al., 2000).

In human, posttranscriptional alternative splicing results in five DDR1 isoforms (a–e), which differ in the intracellular region. DDR1c encodes the full-length transcript, whereas the DDR1b isoform lacks 6 amino acids in the tyrosine kinase domain correlating to the first 18 nucleotids of exon 14. Additional alternative splicing in the juxtamembrane region results in isoforms a, d and e. DDR1a lacks exon 11 and DDR1d exons 11 and 12. In DDR1e, the first half of exon 10 as well as exons 11 and 12 are missing (Alves et al., 2001). The alternative splicing of *Ddr1* in rat and mouse has not been examined in detail so far. Besides the full-length transcript, only one transcript that lacks exon 11 and corresponds to human DDR1a has been described in both species (Sakuma et al., 1995; Perez et al., 1996).

The rat *Ddr1* gene is located on chromosome 20p12 within the rat MHC, the RT1 complex (Günther and Walter, 2001). On the basis of ongoing MHC expression profiling experiments in the rat (Dressel et al., 2001), we analysed *Ddr1* expression and alternative splicing in detail and report here that a testis-specific transcript of *Ddr1* lacking exons 1 to 5 is expressed in a RT1-dependent manner. Furthermore, we identified two novel exons in the 5′-UTR of *Ddr1*.

2. Material and methods

2.1. Rat strains

Rats (*Rattus norvegicus*) of the strains LEW ($RT1^l$), BN ($RT1^n$), DA ($RT1^{av1}$), BUF ($RT1^b$), BB/OK ($RT1^u$) and inbred RT1 congenic strains LEW.1N ($RT1^n$), LEW.1A ($RT1^a$), LEW.1AV1 ($RT1^{av1}$), were bred in the central animal facility of the Medical Faculty, University of Göttingen.

2.2. Northern blot analysis

RNA was prepared according to Chomczynski and Sacchi (1987) from organs frozen in liquid nitrogen immediately after preparation. Northern blots were obtained and hybridized with [^{32}P]dCTP-labeled probes as described (Dressel et al., 1998). Autoradiograms were scanned for densitometry (Epson GT-8000 Scanner, ScanPack software, Biometra, Göttingen, Germany).

2.3. Gene probes for Northern blot hybridization

DNA probes were generated by PCR using BN or LEW.1N-derived cDNA as template. Specific oligonucleotides (Roth, Karlsruhe, Germany) were designed according to the MHC sequence of the rat (GenBank accession no. BX511170). An exon 1 to 4-specific fragment was amplified with the primers 5′-GTGACAATTGGAGATGCTGA-3′ (forward, exon 1) and 5′-AGAGGCAGCCATAGAGCT-3′ (reverse, exon 4). The exon 4 to 5-specific probe was generated by PCR with primers 5′-GTGATTTCGGGTAACGAG-3′ (forward, exon 4) and 5′-ACCAGGCAGCCAATGAGGAT-3′ (reverse, exon 9) followed by a *Sac*I and *Pst*I (Promega, Mannheim, Germany) digestion. The fragment with a length of 126 bp corresponds to the exons 4 and 5. To obtain an exon 1 to 17 specific probe we mixed DNA fragments encompassing exons 1 to 9 and 9 to 17. The primers were 5′-GTGACAATTGGAGATGCTGA-3′ (forward, exon 1) and 5′-ACCAGGCAGCCAATGAGGAT-3′ (reverse, exon 9) as well as 5′-ATCCTTCTCCTCCTCCTTATC-3′ (forward, exon 9) and 5′-TGTTGAGTGCATCATCCGCC-3′ (reverse, exon 17). For the exon 6-specific probe we used the primers 5′-GCTGCATGACGGTGGTCT-3′ (forward, exon 6) and 5′-CCGAGATGAAAGAGATCTCA-3′ (reverse, exon 7). After *Sac*I digestion of the PCR product a 196 bp fragment represents exon 6. The exon 11-specific probe was obtained by using the primers 5′-CGTTGCTGCTCTCCAATCCG-3′ (forward, exon 11) and 5′-CCTGGGTGTTGGTGGGTTTG-3′ (reverse, exon 11). For the exon −1-specific probe the primers 5′-TGCCAGACTTTCCAGTCCCA-3′ (forward, exon −1) and 5′-CAAGGTAGCAGAAGAGCCCA-3′ (reverse, exon −1) were used. A probe containing exons 15 to 17 was obtained from the IMAGE clone UI_p953M2435 (German Resource Center, Berlin, Germany) by *Eco*RI and *Hin*dIII digestion (Fermentas, St. Leon-Rot, Germany).

2.4. cDNA synthesis

For cDNA synthesis 2 μg of total RNA were incubated with 100 pmol of a nonamer random primer (Roth) in a volume of 16 μl at 70 °C for 5 min before being cooled on ice. Reverse transcription was performed for 1 h at 42 °C using 5 μl of 5× reaction buffer (Promega), 1 mM dNTPs (Invitrogen, Karlsruhe, Germany), 40 U RNase inhibitor (Invitrogen), and 200 U reverse transcriptase (Promega) in a final volume of 25 μl.

2.5. PCR and RT-PCR

PCR was performed to generate probes for Northern blot hybridization and to amplify intron 5 using primers 5′-ACTCTTGTCCTATACAGCCCC-3′ (forward, exon 5) and 5′-CTGCATGGTCTGGAAAGACCT-3′ (reverse, exon 6) for subsequent sequencing. RT-PCR was carried out to verify the expression of exon −2 using the specific primers 5′-GGGGCTGAGAGGCTCAGAGA-3′ (forward, exon −2) and 5′-ACCAAGAGTAGCAGCAGCAG-3′ (reverse, exon 1). RT-PCR reactions were performed with primers 5′-ATCCTTCTCCTCCTCCTTATC-3′ (forward, exon 9) and 5′-ATCTTGAGGGTCCTCTACTTCA-3′ (reverse, exon 13) to determine the presence of exon 11 in *Ddr1* transcripts. For PCR and RT-PCR about 5 ng of genomic DNA or cDNA and specific primers (25 μmol each) were mixed with the reaction buffer, $MgCl_2$, dNTPs and Taq DNA polymerase (Invitrogen) according to manufacturer's instructions. The reaction mix was subjected to 35 amplification cycles in a Biometra personal thermocycler (Göttingen, Germany). The