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Expression of T cell receptor alpha gene (TCRA) in human rhabdomyosarcoma and other musculo-skeletal sarcomas

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Abstract

Expression of the T cell receptor (TCR) genes is not restricted to T lymphocytes. Human prostate and breast express a truncated TCR gamma transcript. In the mouse, TCR alpha (TCRA) and beta partial transcripts are expressed by mesenchymal cells and TCRA transcripts by epithelial cells of the kidney. We show now that TCRA constant region expression is common in normal and neoplastic human cells of mesenchymal and neuroectodermal origin. TCR transcripts are derived from an unrearranged TCRA locus. Moreover, rhabdomyosarcoma cells highly expressed a specific J49–C splicing product deriving from the assembly of J49 segment and constant region. TCRA ectopic transcripts/proteins negatively regulate rhabdomyosarcoma cell growth as suggested by TCRA gene expression downmodulation effects using a specific duplex small interfering RNA.

Keywords: Ectopic expression; Mesenchymal cells

1. Introduction

It is generally assumed that expression of T cell receptor (TCR) and immunoglobulin (Ig) genes is confined to the eponymous T and B lymphocytes; however, an alternative protein called TARP (T cell receptor gamma-chain alternate reading frame protein) was found in human prostate cells and in other tissues of epithelial origin (Essand et al., 1999; Wolfgang et al., 2000), and IgG production was reported in human carcinomas and in normal lung tissues (Qiu et al., 2003).

TARP mRNA is transcribed from a non-rearranged TCR gamma (TCRG) gene in normal and neoplastic prostate cells under androgenic hormonal control (Cheng et al., 2003) and in breast cancer cells. Therefore, the control of TARP expression in the prostate is completely different from that of TCR genes in T cells, in which expression follows a

Abbreviations: TCR, T cell receptor; TCRA, T cell receptor alpha; TCRB, T cell receptor beta; TCRG, T cell receptor gamma; Ig, immunoglobulin; TARP, T cell receptor gamma-chain alternate reading frame protein; siRNA, small interfering RNA; HUVEC, human umbilical vein endothelial cells; PBMC, peripheral blood mononuclear cells; RT-PCR, reverse transcriptase polymerase chain reaction; cDNA, DNA complementary to RNA; C, constant; J, joining; V, variable; bp, base pair; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; SLIC-PCR, single strand ligation to ss-cDNA polymerase chain reaction; ss-cDNA, single-stranded cDNA; PEG, polyethylene glycol; PHA, phytohemagglutinin; SDS, sodium dodecyl sulfate; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol-bis(2-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; BM, bone marrow; Fibro, fibroblasts; ORF, open reading frame; RMS, rhabdomyosarcoma; EWS, Ewing's sarcoma; NB, neuroblastoma; GLB, glioblastoma; OSA, osteosarcoma; Ca, carcinoma.

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genetic rearrangement that is unique to T and B cell receptors (Schlissel, 2003). The precise function of TARP is unknown, but some lines of evidence suggest that it might be indirectly involved in the control of cell growth (Wolfgang et al., 2001). Moreover, TARP-specific $CD8^+$ T cells were recently identified in prostate cancer patients, thus raising the possibility of using TARP epitopes as cancer vaccines (Oh et al., 2004; Carlsson et al., 2004).

The human genome contains four TCR genes, A, B, G and D; thus, one could ask whether genes other than TCRG also give rise to alternate transcripts and proteins in non-lymphoid cells. In the mouse, it has been shown that transcripts conceptually and functionally similar to TARP originate from the TCRA and TCRB gene in mesenchymal cells (Barda-Saad et al., 2002). Similar findings were reported for mouse cells of epithelial (kidney) origin (Madrenas et al., 1992, 1994).

At present, it is not known whether in humans the *TCRA* gene gives origin to alternate transcripts and proteins outside the immune system. We show here for the first time that *TCRA* transcripts are common in normal and neoplastic human cells of mesenchymal and neuroectodermal origin, and that a specific splicing product is highly expressed in human rhabdomyosarcoma. Moreover, TCRA ectopic expression seems to negatively regulate rhabdomyosarcoma cell growth.

2. Materials and methods

2.1. Cells

The expression of *TCRA* gene was investigated in human tumor cell lines of mesenchymal and epithelial origin and in normal cells. RD/18, RD/12 and CCA cell lines were derived from embryonal rhabdomyosarcoma (Lollini et al., 1991; De Giovanni et al., 1989), while RMZ-RC2, SJRH30 and SJRH4 cell lines were derived from alveolar rhabdomyosarcoma (Nanni et al., 1986; Shapiro et al., 1993). Osteosarcoma (Saos-2, U-2 OS, MG-63), Ewing's sarcoma (SK-ES, RD-ES), glioblastoma (U373-MG, U87-MG), breast (MDA-MB-453, MCF-7), ovary (SK-OV-3, OAW) and colon carcinoma (LOVO, HT-29) cell lines, the Askin's tumor cell line SK-N-MC, bladder carcinoma (5637), hepatoma (HEPG2), neuroblastoma (SK-N-SH) indicated as HTB11 cell lines, Jurkat T lymphocytes and MRC-5 fibroblasts were obtained from the American Type Culture Collection (Manassas, VA). Other osteosarcoma cell lines (MOS, SARG, OS-7, OS-9, OS-10, OS-14, OS-15, OS-18, OS-20) and the peripheral neuroectodermal tumor cell line LAP-35 were established at the Rizzoli Institute (Bologna, Italy) (Ferracini et al., 1995; Bagnara et al., 1990). TC-71 and 6647 Ewing's sarcoma cell lines were gift from Timothy J. Triche (Children's Hospital, Los Angeles, CA). TS12 and AF8 cell lines were derived from neuroblastoma at the Department of Pediatrics (University of Bologna, Italy). Umbilical vein endothelial cells (HUVEC) were obtained from Cambrex Bio Science (Milan, Italy). 1605 fibroblast cell line was established at the Institute of Histology from a sample of fetal chorionic villi. Bone marrow fibroblasts were obtained from bone marrow samples (obtained after informed consent) by density centrifugation in Ficoll-Hystopaque 1.077 (Sigma, St. Louis, MO) and selecting mononuclear adherent cells by culture in IMDM plus 10% FBS supplemented with recombinant human basic fibroblast growth factor (Invitrogen, Milan, Italy) (0.1 ng/ml) as previously reported (Bonsi et al., 1993). At least four culture passages were performed, by which time macrophagic, endothelial and reticular cells were virtually absent. Peripheral blood mononuclear cells (PBMC) were obtained by density gradient separation and were cultured in IMDM supplemented with 10% FBS and 1% phytohemagglutinin (PHA) (Difco, Detroit, MI).

2.2. RT-PCR and PCR on genomic DNA

Total RNA was extracted from cultured cells by Tri-Zol reagent (Invitrogen) and 1 μg was reverse-transcribed using Moloney murine leukemia virus reverse transcriptase and oligo-dT (Invitrogen). cDNA was amplified using Taq Platinum DNA polymerase or Taq Promega in the presence of 1.5 mM $MgCl_2$, dNTP (0.2 μM each) and specific primer pairs (0.2–0.5 μM each). To amplify the TCRA constant (C) region, primer sequences were derived from Oksenberg et al. (dir: 5′-CCAAATATCCAGAACCCTGACCCT-3′, specific for the C gene first exon; rev: 5′-TGACAGGTTTT-GAAAGTTTAGGTT-3′, specific for the C gene third exon) (Oksenberg et al., 1989). To amplify the J49–C transcript, a primer specific for the joining segment 49 (dir: 5′-GGACAAGTTTGACGGTCATTCC-3′) was used whereas to analyse the expression of the completely rearranged *TCRA* gene a primer specific for the leader sequence (dir: 5′-ATGCTCCTGCTGCTCGTCCCA-3′) was used. These two primers were used coupled with the previous rev primer and were designed with Amplify program (http://engels.genetics.wisc.edu/amplify/). Products of 351 bp (for the C transcript), 373 bp (for the J49–C transcript) and 756 bp (for the fully rearranged *TCRA* gene) indicated a correct process of splicing/rearrangement and excluded the amplification of genomic DNA (GenBank accession number M94081). The amplification of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH, Clontech, Palo Alto, CA) was carried out to ensure that comparable amounts of cDNA were analysed.

To analyse whether the J49-C transcript derives from a genomic pathological rearrangement, 0.1 μg of DNA extracted from RD/18, HT-29 and Jurkat cells by Tri-Zol reagent was amplified using the J49-specific primer and the C gene, third exon, reverse primer (expected product in case of genomic rearrangement: 3131 bp). A DNA polymerase suitable for the amplification of long DNA products was used (AccuPrime DNA Polymerase, Invitrogen). The annealing temperature was 60 °C for all PCR reactions.