Absence of interaction between doppel and GFAP, Grb2, PrPc proteins in human tumor astrocytic cells.

Azzalin A, Del Vecchio I, Chiarelli LR, Valentini G, Comincini S, Ferretti L.

Dipartimento di Genetica e Microbiologia, Universita di Pavia, via Ferrata 1, 27100 Pavia, Italy. alberto@ipvgen.unipv.it

BACKGROUND: The doppel protein (Dpl) is a newly recognized cellular prion protein (PrP(C))-like molecule encoded by a novel gene locus, PRND, located on the same chromosomal region of the PrP(C) coding gene. Recently, Dpl was shown to be aberrantly expressed in astrocytic tumor specimens and in astrocytoma-derived cell lines, showing a peculiar cytoplasmic localization. Here, Dpl interactions with some of the prion-interacting proteins were studied. In particular, whether the tumor astrocytic environment is suitable for doppel interaction with GFAP and Grb2 proteins, as well as with the PrPC protein itself was investigated. MATERIALS AND METHODS: In order to verify our hypothesis, an innovative mammalian two-hybrid system

Related Links


Abnormal activation of glial cells in the brains of prion protein-deficient mice ectopically expressing prion protein-like protein, PrPLP/Dpl.

Human Doppel and prion protein share common membrane microdomains and internalization pathways.
and co-immunoprecipitation assays were employed. RESULTS: The results reported the absence of protein interactions. Our findings provided evidence that, in our astrocytoma cell-based model, Dpl does not share with PrP(C) the ability to interact with GFAP and Grb2. CONCLUSION: Identifying Dpl ligands may provide new insights into the involvement of Dpl in astrocytoma tumor progression.

PMID: 16309242 [PubMed - indexed for MEDLINE]


Dipartimento di Genetica e Microbiologia, Universita di Pavia, Pavia, Italy. sergio.c@ipvgen.unipv.it

The expression of the prion (PRNP) and prion like-doppel (PRND) genes and the presence of the proteins prion (PrP) and doppel (Dpl) were investigated in human gliomas. The PRNP and PRND expression profiles were evaluated by real-time reverse transcription-quantitative PCR in low- and high-grade astrocytomas, in glioblastoma-derived cell lines and in non-glial tumor specimens. The presence of PrP and Dpl proteins and their cellular localization were evaluated by Western blot and immunohistochemistry. High levels of PRNP expression were found in all tumoral samples studied. Unlike the non-tumoral controls, PRND was aberrantly expressed in glioblastoma multiforme and in two glioblastoma multiforme-derived cell lines, even in the absence of the PRND gene amplification. PRND expression was directly related to malignancy of the tumor: highest in glioblastoma multiforme, lower in anaplastic astrocytoma and even lower in the low-grade astrocytoma samples. High levels of PRND were also found in non-glial malignant tumor samples, such as gastric adenocarcinoma and anaplastic meningioma. Western blot analysis confirmed the PrP and Dpl expression, displaying variability in the electrophoretic patterns. Immunohistochemical analysis revealed a diffuse cytoplasmatic Dpl distribution in different astrocytic neoplastic cells, in infiltrating lymphocytes and in blood vessel endothelial cells. Of note, Dpl reactivity was different from that of the PrP, since PrP showed typical Golgi and membrane localised staining. Our findings suggest that the PRND gene might be a useful molecular marker in astrocytoma progression and in tumor grade definition. Understanding of the mechanisms of PRND increased expression might provide insight into the regulatory pathways of glioma development.
Searching for molecular markers of human gliomas.

Comincini S.

Laboratory of Experimental Neurobiology, IRCCS C. Mondino Institute of Neurology, Maugeri-Mondino-University of Pavia Research Center, Italy. sergio.c@ipvgen.unipv.it

PMID: 11996527 [PubMed - indexed for MEDLINE]
Identification of genetic markers of the glial tumor by means of differential display technique.

Comincini S, Capelli E, Cattana E, Zanoli E, Ceroni M, Nano R.

Istituto Neurologico C. Mondino, Pavia, Italy.

The Differential Display Reverse Transcriptase (DDRT) technique was adopted to isolate genetic markers specific for the two main grades of the Glial tumor, the Astrocytoma and the Glioblastoma. A total of 16 brain biopsies (4 Astrocytoma and 12 Glioblastoma) were analysed. The technique was modified in order to reduce the false-positive ratio by means of more stringent amplification conditions. Electrophoretic patterns with previously selected arbitrarily primers revealed differences between the grades, four of them were investigated through sequencing. These sequences did not show significant nucleotide and aminoacid similarity to known sequences in the Database. Sensitivity of the method was documented by the evidence that only one of the selected markers was an artefact, while the others represented genetic markers of the human Glial neoplasm.

PMID: 10226554 [PubMed - indexed for MEDLINE]
Interaction between the cellular prion (PrPC) and the 2P domain K+ channel TREK-1 protein.


Dipartimento di Genetica e Microbiologia, Universita di Pavia, Pavia, Italy.

The cellular prion protein (PrP(C)) is a highly conserved protein throughout the evolution of mammals and therefore is thought to play important cellular functions. Despite decades of intensive researches, the physiological function of PrP(C) remains enigmatic. Differently, in particular pathological contexts, generally referred as transmissible spongiform encephalopathies, a conformational isoform of PrP(C), i.e., PrP(Sc), is considered the causative agent of these diseases. In this study, we investigated putative PrP(C) cellular functions through the identification of PrP(C) protein interactants. Using a bacterial two-hybrid approach, we identified a novel interaction between PrP(C) and a two-pore potassium channel protein, TREK-1. This interaction was further verified in transfected eukaryotic cells using co-immunoprecipitation and confocal microscopic analysis of the fluorescent transfected proteins. Importantly, in the cerebellar cortex, the endogenous PrP(C) and TREK-1 proteins exhibited co-localization signals in correspondence of the Purkinje cells. Furthermore, a deletion mapping study defined the carboxyl-terminal regions of the two proteins as the possible determinants of the PrP(C)-TREK-1 interaction. Our results indicated a novel PrP(C) interacting protein and suggested that this complex might be relevant in modulating a variety of electrophysiological-dependent cellular responses.

PMID: 16750514 [PubMed - indexed for MEDLINE]
Application of quantitative real-time PCR in the detection of prion-protein gene species-specific DNA sequences in animal meals and feedstuffs.

Bellagamba F, Comincini S, Ferretti L, Valfre F, Moretti VM.

Dipartimento di Scienze e Tecnologie Veterinarie per la Sicurezza Alimentare (VSA), Facolta di Medicina Veterinaria, Universita degli Studi di Milano, Italy. federica.bellagamba@unimi.it

This study describes a method for quantitative and species-specific detection of animal DNA from different species (cattle, sheep, goat, swine, and chicken) in animal feed and feed ingredients, including fish meals. A quantitative real-time PCR approach was carried out to characterize species-specific sequences based on the amplification of prion-protein sequence. Prion-protein species-specific primers and TaqMan probes were designed, and amplification protocols were optimized in order to discriminate the different species with short PCR amplicons. The real-time quantitative PCR approach was also compared to conventional species-specific PCR assays. The real-time quantitative assay allowed the detection of 10 pg of ruminant, swine, and poultry DNA extracted from meat samples processed at 130 degrees C for 40 min, 200 kPa. The origin of analyzed animal meals was characterized by the quantitative estimation of ruminant, swine, and poultry DNA. The TaqMan assay was used to quantify ruminant DNA in feedstuffs with 0.1% of meat and bone meal. In conclusion, the proposed molecular approach allowed the detection of species-specific DNA in animal meals and feedstuffs.

PMID: 16629035 [PubMed - indexed for MEDLINE]
Prion-like Doppel gene (PRND) in the goat: genomic structure, cDNA, and polymorphisms.


Dipartimento di Genetica e Microbiologia, Università di Pavia, via Ferrata 1, 27100, Pavia, Italy.

The genomic structure of the caprine Doppel gene (PRND) was determined using the ovine sequence as a scaffold to generate PCR fragments that were aligned with a cDNA sequence obtained from testicular mRNA. The caprine gene contains two exons, 89 and >2291 bp long, separated by a 1689-bp intron. Two mRNA isoforms of 3.2 and 4.8 kb were identified in the testis, as well as the exact transcription start site by fluorescently labeled oligonucleotide extension (FLOE). Like in sheep and cattle, the open reading frame (ORF) (537 bp) lies within exon 2 and is very much conserved in sheep (99.3%) and cattle (97%). The intron sequence is also highly conserved (95.3%) compared with sheep, with the only exception of a 47-bp insertion. The PRND ORF was sequenced in 47 healthy and 17 TSE-affected goats of the Italian Ionica breed. Seven nucleotide positions showed variation: T28C, C65T, A151G, G286A, C385G, T451C, and T528C. Five were commonly represented polymorphisms: T28C, T451C, and T528C are silent mutations at codons L10, L151, and I176, respectively, while A151G and C385G determine a T51A and L129V amino acid change, respectively. The two remaining variants, C65T and G286A, were rare, leading to the amino acid substitutions S22F and E96K, respectively. None of the polymorphisms was significantly relatable to the TSE status, and the same result was obtained by the analysis of the combined haplotypes at the five major polymorphic sites, namely, T28C, C65T, A151G, G286A, and C385G.

PMID: 16341676 [PubMed - indexed for MEDLINE]

**Related Links**

- Genomic organization, comparative analysis, and genetic polymorphisms of the bovine and ovine prion Doppel genes (PRND). [Mamm Genome. 2001]
- Isolation and molecular characterization of the porcine stearoyl-CoA desaturase gene. [Gene. 2004]
- Analysis, identification and correction of some errors of model refseqs appeared in NCBI Human Gene Database by in silico cloning and experimental verification of novel human genes. [Yi Chuan Xue Bao. 2004]
- The PrP-like protein Doppel gene in sheep and cattle: cDNA sequence and expression. [Mamm Genome. 2001]
Absence of interaction between doppel and GFAP, Grb2, PrPc proteins in human tumor astrocytic cells.

Azzalin A, Del Vecchio I, Chiarelli LR, Valentini G, Comincini S, Ferretti L.

Dipartimento di Genetica e Microbiologia, Università di Pavia, via Ferrata 1, 27100 Pavia, Italy. alberto@ipvgen.unipv.it

BACKGROUND: The doppel protein (Dpl) is a newly recognized cellular prion protein (PrP(C))-like molecule encoded by a novel gene locus, PRND, located on the same chromosomal region of the PrP(C) coding gene. Recently, Dpl was shown to be aberrantly expressed in astrocytic tumor specimens and in astrocytoma-derived cell lines, showing a peculiar cytoplasmic localization. Here, Dpl interactions with some of the prion-interacting proteins were studied. In particular, whether the tumor astrocytic environment is suitable for doppel interaction with GFAP and Grb2 proteins, as well as with the PrPC protein itself was investigated. MATERIALS AND METHODS: In order to verify our hypothesis, an innovative mammalian two-hybrid system and co-immunoprecipitation assays were employed. RESULTS: The results reported the absence of protein interactions. Our findings provided evidence that, in our astrocytoma cell-based model, Dpl does not share with PrP(C) the ability to interact with GFAP and Grb2. CONCLUSION: Identifying Dpl ligands may provide new insights into the involvement of Dpl in astrocytoma tumor progression.

PMID: 16309242 [PubMed - indexed for MEDLINE]


Dipartimento di Genetica e Microbiologia, Università di Pavia, Pavia, Italy. sergio.c@ipvgen.unipv.it

The expression of the prion (PRNP) and prion like-doppel (PRND) genes and the presence of the proteins prion (PrP) and doppel (Dpl) were investigated in human gliomas. The PRNP and PRND expression profiles were evaluated by real-time reverse transcription-quantitative PCR in low- and high-grade astrocytomas, in glioblastoma-derived cell lines and in non-glial tumor specimens. The presence of PrP and Dpl proteins and their cellular localization were evaluated by Western blot and immunohistochemistry. High levels of PRNP expression were found in all tumoral samples studied. Unlike the non-tumoral controls, PRND was aberrantly expressed in glioblastoma multiforme and in two glioblastoma multiforme-derived cell lines, even in the absence of the PRND gene amplification. PRND expression was directly related to malignancy of the tumor: highest in glioblastoma multiforme, lower in anaplastic astrocytoma and even lower in the low-grade astrocytoma samples. High levels of PRND were also found in non-glial malignant tumor samples, such as gastric adenocarcinoma and anaplastic meningioma. Western blot analysis confirmed the PrP and Dpl expression, displaying variability in the electrophoretic patterns. Immunohistochemical analysis revealed a diffuse cytoplasmatic Dpl distribution in different astrocytic neoplastic cells, in infiltrating lymphocytes and in blood vessel endothelial cells. Of note, Dpl reactivity was different from that of the PrP, since PrP showed typical Golgi and membrane localised staining. Our findings suggest that the PRND gene might be a useful molecular marker in astrocytoma progression and in tumor grade definition. Understanding of the mechanisms of PRND increased expression might provide insight into the regulatory pathways of glioma development.
Identification of genetic markers of the glial tumor by means of differential display technique.

Comincini S, Capelli E, Cattana E, Zanoli E, Ceroni M, Nano R.

Istituto Neurologico C.Mondino, Pavia, Italy.

The Differential Display Reverse Transcriptase (DDRT) technique was adopted to isolate genetic markers specific for the two main grades of the Glial tumor, the Astrocytoma and the Glioblastoma. A total of 16 brain biopsies (4 Astrocytoma and 12 Glioblastoma) were analysed. The technique was modified in order to reduce the false-positive ratio by means of more stringent amplification conditions. Electrophoretic patterns with previously selected arbitrarily primers revealed differences between the grades, four of them were investigated through sequencing. These sequences did not show significant nucleotide and aminoacid similarity to any known sequences in the DataBase. Sensitivity of the method was documented by the evidence that only one of the selected markers was an artefact, while the others represented genetic markers of the human Glial neoplasm.

PMID: 10226554 [PubMed - indexed for MEDLINE]