DOPACHROME TAUTOMERASE – AN OLD PROTEIN WITH NEW FUNCTIONS

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Dopachrome tautomerase (DCT) was first described as an enzyme acting at one of the distal steps in melanogenesis. Within the last decade there is growing evidence for DCT participation in additional cellular processes. Here we review the present status of DCT, including structural characteristics and regulatory mechanisms which control its cellular expression, outline the novel DCT-mediated pathways in tumor resistance to environmental and therapeutic stress, and discuss the future of DCT in melanoma or glioma therapy and diagnosis.

Key words: Tyrosinase related protein-2, dopachrome tautomerase, melanogenesis, melanoma, immunotherapy, tumor cell resistance.

HISTORICAL BACKGROUND

For many years it was considered that melanin synthesis pathway was regulated only by enzyme tyrosinase (Tyr), which converts tyrosine into DOPA and further to DOPAquinone. All subsequent steps culminating with the formation of melanin polymer were thought to occur spontaneously without participation of other enzymatic components. Early studies about the by-products of melanin synthesis identified a factor termed DOPAchrome conversion factor (1, 2) or DOPAchrome oxidoreductase (3) or DOPAchrome isomerase which was capable to convert rapidly DOPAchrome from red orange to a colourless compound (4). In 1992, Jackson *et al.* reported the cloning and sequencing of mouse cDNA corresponding to the region of the coat color mutation *slaty* (5). The product of this gene named Tyrosinase Related Protein-2 (TRP-2) due to its high degree of amino acid identity with the other two proteins, tyrosinase and tyrosinase related protein had DOPAchrome tautomerase activity (6). In different extracts of *slaty* tissues, the

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DOPAchrome tautomerase activity of TRP-2 was severely diminished, indicating that *slaty* locus indeed encodes the TRP-2/DCT.

DCT AS MEMBER OF TRP FAMILY-STRUCTURAL CHARACTERISTICS

The pigmentation in mammals is a complex process which in mouse is regulated by over 100 genes. The most studied are three loci which encode the enzymes that control the main steps in melanin formation, tyrosinase (Tyr), Tyrosinase Related Protein-1 (TRP-1) and Tyrosinase Related Protein-2 (TRP-2) or DCT. The analysis of the TRP sequences revealed a high degree of homology among the three proteins of this family. The aminoacid sequences showed 50% identity between TRP-1 and TRP-2 and 40% identity with Tyr (7, 8). TRPs are type I membrane proteins and their polypeptide is organized after a common pattern. An amino terminal signal sequence, important for the import of TRP chain into the endoplasmic reticulum (ER) compartment and correct folding; the 15 conserved cysteine residues involved in disulfides formation which ensure the functional conformation of the active protein; two metal binding domains containing 3 conserved histidine residues required for metal coordination and enzymatic activity; the potential N-glycosylation sites involved in the interaction with ER lectin chaperones during polypeptide folding; a hydrophobic region which anchors TRP chain in membranes of melanosomes or different endosomal vesicles during intracellular trafficking; the cytoplasmic tail in the carboxy terminal of the protein which interacts with the elements of the sorting and traffic machinery.

Despite these general characteristics each TRP displays particularities. In case of DCT, the metal in the active catalytic site is Zn^{++}. Purified DCT contains 2 Zn atoms per protein molecule as measured by atomic absorption spectroscopy (9). The enzyme DCT reconstituted with Co^{++} instead of Zn^{++} reached more than 60% of the activity further supporting that DCT is a Zn-enzyme (10). It should be noted that human and mouse DCT contain 17 and 16 cysteine residues, respectively, excluding the two residues in each signal peptide (11). The molecular weight of murine DCT of approximately 82 kDa by SDS-PAGE and the glycolytic digestion indicated that mature DCT contains significant amounts of complex sialylated N-linked oligosaccharides (12) compared to TRP-1 and Tyr (13). The carboxy terminal domain of mouse or human DCT has 30 amino acids, whereas the same domains in TRP-1 or Tyr contain 41 amino acids. Interestingly, there is practically no homology in the transmembrane and cytosolic domains of TRPs. The di-Leu motif (QPLLMD) is present in both cytoplasmic tails of Tyr and TRP-1 and is specifically involved in the interaction with the AP-3/ AP-1 sorting elements, but it is absent from DCT cytoplasmic domain which has tyr-like motif (YRRL) (14). The di-Leu motif, which is also identified in lysosomal membrane proteins,

provides evidence for a similar sorting mechanism and pathways for lysosomal and melanosomal proteins, and may suggest that DCT could be trafficked on a distinct route than Tyr or TRP-1. Unlike for Tyr and TRP-1, no mutations have been described in human gene of DCT, suggesting this is a conserved protein. However, in mouse several mutant alleles of DCT are associated with pigment dilution. Mutant DCT produced by *slaty* mice has a single amino acid difference R194Q in the first metal binding domain. Another point mutation in the exon 8 was identified in the DCT gene of *slaty* mice named *slaty light* (15), which results in a G486R substitution in the transmembrane domain. Chemical analysis showed that both DCT mutations increase pheomelanin and reduce eumelanin produced by melanocytes in culture, suggesting that enzymatic activity of DCT may play a role in determining whether pheo-or eu-melanin pathway is preferred (16).

DCT CELLULAR EXPRESSION

DCT in melanocytes – Like other proteins involved in melanin synthesis, DCT is expressed mainly in melanocytes, the cells that produce melanin pigment. Despite the fact that melanocytes populate tissues of different organs, such as, skin, hair, eyes, ear, it is not known whether melanogenic proteins have structural or functional particularities within a specific tissue. The melanocytes which originate from neural crest (NC) migrate during embryonic development to different regions, such as epidermis and hair follicles, retina and choroid of the eye, the inner ear and Harderian gland. In addition, some melanocytes of the retinal pigment epithelium (RPE) originate from the forebrain neuroepithelium. A recent study of Jiao *et al*. (17) reported that mouse neural crest derived melanocytes have the DCT transcript different from that of RPE. There are 9 exons encoding DCT and the alternative splicing of exon 7 results in two isoforms of DCT transcripts. The DCT transcript which lacks exon 7 is present in the mouse brain and neural-crest derived melanocytes, whereas the DCT transcript, that lacks exons 8 and 9, is present in RPE melanocytes. The identity of DCT isoforms in cells with common origin may also indicate similarities in pathways or processes regulated by DCT in these tissues; however, no experimental evidence has been provided to support this so far. Multiple isoforms of DCT mRNA, which were generated postranscriptionally by alternative poly (A) site usage or by alternative splicing of DCT mRNA, have been described in melanocytes and melanoma cells, suggesting that they might play a part in the normal pathway of melanin biosynthesis (18).

DCT in cells of nervous system – In addition to NC-derived melanocytes, precursors of peripheral nervous system derive also from NC. Recent studies related to neurogenesis in development demonstrated that spatial and temporal profiles of DCT expression correlate with neurogenesis during embryonic

development (17). Overexpression of DCT enhanced the proliferation of cortical neural progenitor cells, whereas silencing DCT by siRNA blocked the same process. All together, these data indicate that DCT regulates proliferation of neural progenitor cells and contributes thus to maintain the pool of neural progenitor cells. An additional important finding of this study was that DCT is strongly expressed in adult rostal migratory stream, indicating that DCT could regulate neuroblast migration.

REGULATION OF DCT EXPRESSION

Transcriptional level. The coordinated regulation of the TRP family genes is argued by the presence of cis-regulatory element, known as M-box. The possibility that DCT gene is regulated independently of Tyr and TRP-1 genes has been suggested by early investigations. During the mouse embryonic development, DCT gene precedes the activation of Tyr and TRP-1 (19). The expression of DCT gene has also been reported in certain non-melanocytic cells lacking detectable Mitf expression (20). Mitf is a critical factor in the commitment, proliferation and survival of melanocytes during neural crest migration (21) and a lineage specific regulator of major pigment genes including Tyr, TRP-1, Pmel17 (22). In human melanomas, unlike Tyr, DCT promoter is not under direct activation of Mitf (23), whereas in mouse DCT this regulation is direct, which suggests a species-specific mechanism. In a recent study, Schwahn *et al*. demonstrated the distinct regulation of DCT promoter in proliferating and senescent melanocytes by Mitf, estrogen receptor –α (ER-α) and histone acetyltransferase p300 (24). They proposed a model whereby transcription of DCT is highly dynamic and tightly regulated by transcriptional co-activators and by the proliferation status of melanocytes. The absence of Mitf and DCT proteins in senescent melanocytes may result in altered melanin chemistry and increased susceptibility to UVB damaged in aged skin. In addition to Mitf, DCT promoter interacts directly with Sox10. The deletion mutagenesis studies of Jiao *et al*. (25) showed that sequences within proximal 459 nucleotides are critical for high level expression in melanocytic cells. This region of the promoter contains candidate binding sites for transcription factors Sox10 and Mitf. They independently activate Dct expression and when co-transfected, they synergistically activate DCT expression. Treatment of proliferating melanocytes with the differentiation inducing pharmacological agent hexamethylene bisacetamide resulted in distinct change in morphology and up-regulation of DCT, while quiescent melanocytes responded by a dramatic increase in expression of DCT without change in morphology, which suggested an inverse relationship of DCT gene regulatory mechanisms to melanocyte growth regulatory pathways (26).

Posttranslational level. The intracellular processing of TRPs follows the general pathways of type I membrane N-glycosylated proteins. Studies of Petrescu

et al. (27) extensively dissected the folding pathways of TRPs and showed that folding processes of the three mouse TRP-polypeptides are distinctly controlled despite their high degree of homology. All TRP monoglucosylated precursors were found to interact with the ER lectin chaperone calnexin, which assisted the normal polypeptide folding. The inhibition of N-glycan processing in the ER, which prevents TRP-polypeptide to interact with calnexin, resulted in Tyr inactivation, but did not prevent its transport to melanosomes (27) and perturbed TRP-1 folding and maturation (28)**.** Unlike Tyr or TRP-1, the interaction of DCT polypeptide with calnexin is an absolute requirement for the stability of this protein. DCT folded in the absence of calnexin is targeted to proteasomal degradation (12). DCT represents a good model to document the calnexin independent retro-translocation process of proteasomally degraded proteins. Post ER, DCT and TRP-1 maturation pathways along the Golgi complex are also distinctly regulated indicating that in melanoma cells a dual early secretory pathway may operate (29). Post Golgi, DCT stability is specifically controlled by lysomotropic agent chloroquine, which diverts DCT, but not TRP-1, to a degradation pathway. A model for DCT traffic is proposed by which DCT intersects the endocytic pathway following a route *via* early endosomes possibly by rapid recycling from the plasma membrane and this cargo does not include TRP-1 (29). However, the molecular machinery behind the sorting and traffic of DCT has not been identified yet.

DCT BIOLOGICAL FUNCTIONS

DCT enzymatic function. The initial and rate-limiting step in melanin biosynthesis (Fig. 1) is catalyzed by Tyr, which converts L-tyrosine to o-diphenol (L-DOPA) and further oxidizes L-DOPA to L-DOPAquinone. L-DOPAquinone is the first branch point in melanogenesis. Alternatively, in the absence of free thiol groups, L-DOPAquinone slowly cyclates to L-cycloDOPA, which follows a redox reaction when DOPAchrome results. These two reactions are very fast and are not enzymatically assisted. L-DOPAchrome represents the second branch point in melanogenesis. L-DOPAchrome undergoes spontaneous decarboxylation resulting in 5,6-dihydroxyindole (DHI) formation. Alternatively, in the presence of DCT, L-DOPAchrome is converted by a nondecarboxylate rearrangement to 5,6-dihydroxyindole carboxylic acid (DHICA), which is further substrate for TRP-1. Once the product of DCT action was established as DHICA instead of DHI, the name of tautomerase was proposed and accepted, the enzyme being definitively assigned as E.C.5.3.3.12. It is interesting to note that traces of metal ions can yield mixtures of DHI and DHICA, whereas the latter is exclusively the product of the DCT enzymatic reaction (30). Moreover, metal ion-catalyzed DOPAchrome rearrangement is not dependent on the substrate stereospecificity, whereas DCT acts only on the

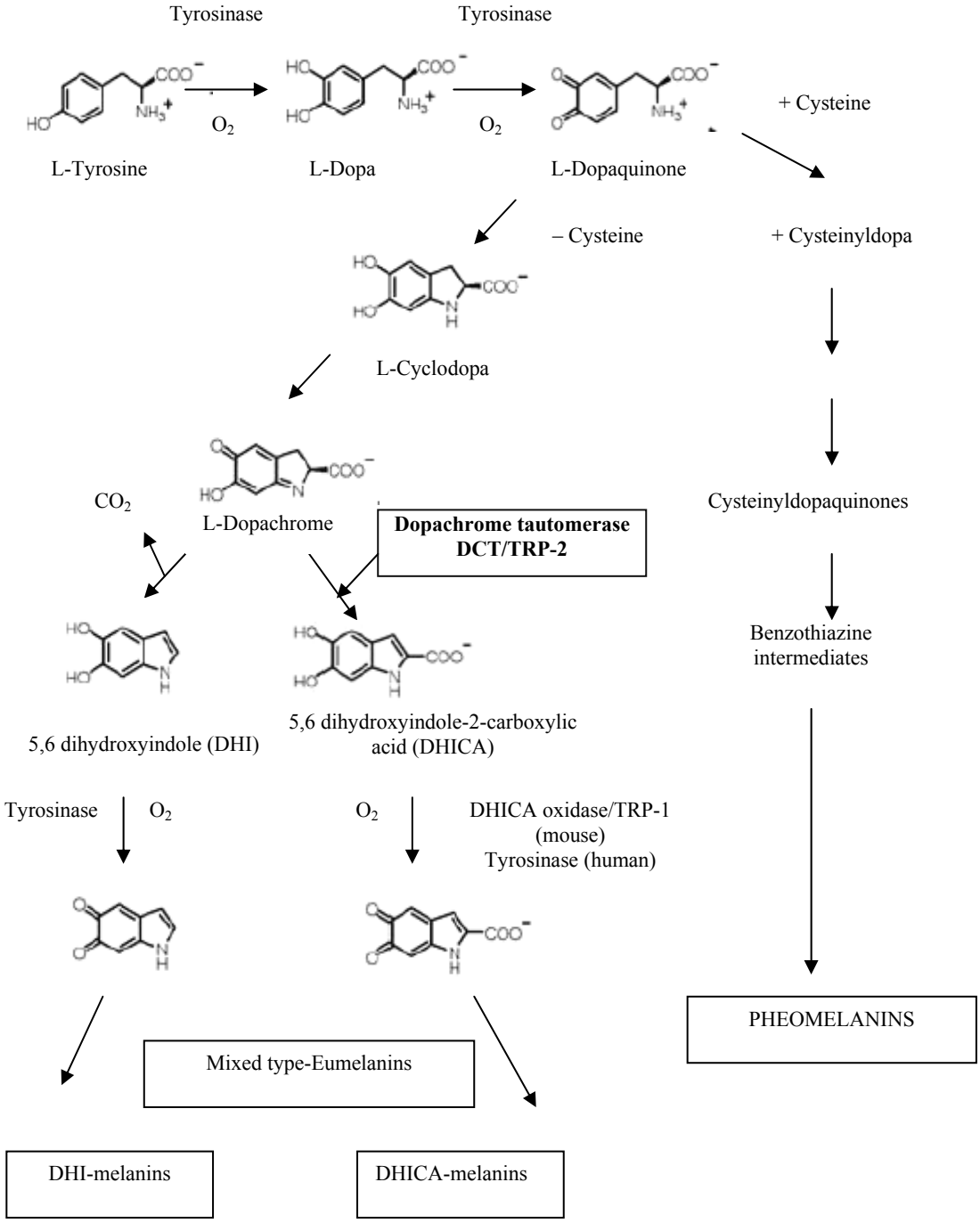

Fig. 1. – The melanogenic pathway.

L-DOPAchrome. The carboxyl group is essential for the docking of substrate at the ezyme active site, as neither D-stereoisomer or decarboxylated dopaminochrome are substrates for DCT (31). These two compounds represent substrates for other DCT-related enzymes, which catalyze decarboxylation instead of tautomerization and their product is DHI instead of DHICA. The presence of Zn^{++} in the catalytic site is in agreement with the DCT tautomerase activity, as Zn^{++} has no redox properties and is unable to catalyze oxidative reactions. The molecular mechanism of DCT-catalyzed tautomerization of DOPAchrome proposed by Solano *et al*. is the following: the active site of DCT, which contains two tetrahedral Zn^{++} ions, would bind one L-DOPAchrome molecule. The absence of redox properties of the metal ion induces the field effects on the substrate that lead to its electronic rearrangement and subsequent migration consistent with tautomerization to DHICA (32). DCT may contribute to the formation of DHICA-enriched melanins, which contain a higher proportion of carboxylated *versus* non- carboxylated indolic monomers. These are brown coloured compared to DHI-rich melanins which are black (33, 34). As DHICA represents a less cytotoxic compound than DHI, DCT may protect melanocytes from the inherent cytotoxic intermediates of melanogenesis (35) and this will be further discussed. The physiological consequences of DCT on the type and amount of melanin formed are insufficiently understood.

DCT in the pathology of the pigmentary and nervous system. In pathological conditions, melanocytes can be either the target of the autoimmune destruction, as in autoimmune depigmentation vitiligo, or can undergo an uncontrolled proliferation and transform into melanoma cells. In the nervous system, a type of cancer related to melanoma is glioma which arises from glial cells and the most common site of gliomas is the brain. Next we will discuss the involvement of DCT as autoantigen or as tumor antigen and the perspectives for DCT to be used in the therapy of these diseases.

DCT as autoantigen in vitiligo. Vitiligo is an acquired hypomelanotic disorder characterized by circumscribed depigmented macules in the skin resulting from the loss of functional melanocytes. However, even if antibodies to melanocytes are not an agent of the disease, identifying their target antigens could provide for the development of diagnostic tests that are not yet available for vitiligo and could serve as markers for important T cell responses in patients with the disease. In vitiligo, TRP-2 is a major autoantigen, as autoantibodies against it were detected in sera of vitiligo patients (36). A correlation study of antigen-specific immune responses in patients with the autoimmune disease vitiligo, therapy-induced hypo-pigmentation, and cutaneous melanoma (37) has shown that patients with malignant melanoma, vitiligo, and active-specific immunotherapy-induced depigmentation had significant anti-TRP-2 IgG titers. The highest level of anti-TRP-2 IgG response was found in vitiligo patients. Induction and enhancement of anti-TRP-2 IgG responses were observed in melanoma patients treated with a polyvalent melanoma cell vaccine

containing TRP-2. Active-specific immunotherapy could induce and/or augment the TRP-2 IgG antibody titers. Melanoma patients who developed hypopigmentation and had improved survival after polyvalent melanoma cell vaccine had significantly augmented anti-TRP-2 antibody responses compared with patients with poor prognosis. This study demonstrates that TRP-2 autoantigen is immunogenic in humans. TRP-2 antibody responses provide a linkage between autoimmune responses by vitiligo patients and melanoma patients responding to immunotherapy which has induced hypopigmentation.

DCT as tumor antigen in melanoma and glioma. Malignant melanoma and glioblastoma multiforme are among the most devastating cancers. It has been acknowledged that malignant melanoma is immunogenic. During the radial growth phase, a dermal lymphocytic infiltrate is seen on histological examination (38) and this represents one criterion to distinguish melanoma from benign nevus. There are rare cases when disseminated melanoma has undergone regression and the pathological examination strongly involves the immune attack through lymphocytes, plasma cells and macrophages resulting in tumor destruction. Various studies have identified as targets of the immune response melanocytic lineage proteins or normal differentiation antigens (MDA) which are involved in melanin synthesis and are expressed in both normal melanocytes and melanoma cells. The most frequent MDAs targeted in HLA-A2 specific vaccination have been MART-1, Tyr and gp100 (39).

The interest for using TRP-2 in vaccination therapy started when certain TRP-2 sequences recognized by T lymphocytes in melanoma patients were identified. The sequences from DCT recognized by different T cell clones from a population of TIL which mediated tumor regression were hTRP-2 $_{197–206}$ peptide (40) or a non-mutated product of mouse TRP-2 gene, TRP-2 $_{181–188}$ (41). The same peptide induced CTLs from patients that specifically recognized peptide pulsed T2 cells, COS cells expressing HLA-A* 0201 and TRP-2 and HLA-$^{A2+}$ TRP-2^{+} melanomas (42), suggesting that TRP2 may be useful for both the development of murine tumor immunotherapy models and the treatment of melanoma patients who are diverse in HLA expression. Another epitope ANDPIFVVL of TRP-2 (387–395) was recognized by CTL restricted by HLA-Cw8. Identification of such additional T cell epitopes presented by alternative HLA-B and -C alleles may provide a means to counteract the tumor escape mechanism based on the selection of tumor cells no longer susceptible to HLA-A-restricted T cell recognition (43). Specific epitopes resulted from alternatively spliced forms of TRP-2 have been identified. The sequence encoded by previously unknown TRP-2 mRNA isoform (TRP-2-6b) contained two novel exons alternatively spliced from the sixth intron between exons 6 and 7 of TRP-2 mRNA (44) or a partially spliced form of TRP-2 containing exons 1–4 with retention of intron 2 and part of intron 4 (TRP-2-INT2)

was associated with CTL response in melanoma (45). Another naturally processed epitope TRP-2 360–368 (TLDSQVMSL) peptide was specifically recognized by clones able to lyse HLA-A2.I+ melanoma cells expressing TRP-2 (46). In addition to the immune response against DCT mediated by $CD8^+$ lymphocytes, TRP-2(60–74) as an HLA-DRB1*0301-restricted Th epitope was identified (47).

Anti-melanoma therapeutic strategies involving DCT. The identification of various DCT epitopes recognized by CD8+ or CD4+ lymphocytes urged the development of numerous immunological therapeutic strategies involving DCT. Coexpressing a tumor antigen epitope, with IFN-γ in the same gene by replacing the IFN-γ signal peptide with a TRP-2 epitope-expressing signal peptide, B16 tumorigenicity decreased and immunogenicity was enhanced after gene transfer (48). The immunization with TRP-2 derived peptides or in combination with other antigens or adjuvants using various delivery vectors have been also developed. The VacciMax® (VM), a liposome-based antigen delivery platform has been used to deliver TRP-2(181–188) in combination with p53-derived peptides. A single administration of VM was capable of inducing an effective CTL response to multiple tumor associated antigens. The responses generated were able to reject 6-day old B16-F10 tumors (49). Another plasmide liposome DNA vaccine targeting the TRP-2 used xenogeneic (human) TRP-2 and chemokine CCL21 as an adjuvant in a mouse model and resulted in induction of strong anti-TRP-2 cell mediated immunity after two vaccinations (50). An interesting study which explored the linkage between melanoma immunotherapy and autoimmunity using vaccination with MDAs showed that inoculation of plasmid DNA encoding murine TRP-2 elicited antigen-specific CTLs that recognized the B16 mouse melanoma and protected the mice from challenge with tumor cells. Moreover, mice that rejected the tumor did not develop generalized vitiligo, indicating that protective immunity can be achieved in the absence of widespread autoimmune aggression (51). Immunization with dendritic cells transfected with genes encoding tumor-associated antigens is a highly promising approach to melanoma immunotherapy. The vaccination with TRP-2 peptide loaded bone marrow derived dendritic cells results in activation of high avidity CTLs mediating protective antitumor immunity *in vivo* without the development of adverse autoimmunity (52).

DCT as immunotherapeutic target in glioma. Melanocytes and astrocytes are both derived embryologically from the neural ectoderm. Their neoplastic counterparts, malignant melanomas and gliomas, have been shown in humans to share common antigens at the RNA level. Different studies provide evidence that glioma cells express TRP-2 and that gliomas TRP-2-specific CD8(+) T cells have been identified which provided further evidence that these gliomas express the protein products in the context of MHC class I (53,54). Moreover, dendritic cells pulsed with TRP-2 and gp100 derived peptides could prime T cells that specifically

recognize GL26 glioma cells *in vitro*. The mice pre vaccinated with human gp100 and TRP-2 peptide-pulsed dendritic cells had significantly extended survival when challenged with tumor cells in the brain, resulting in >50% long-term survival. An additional study showed that after immunization with tumor lysate pulsed dendritic cells, TRP-2 specific CTLs were induced in the peripheral blood of patients who were HLA-A2+ and whose tumors expressed TRP-2 suggesting that TRP-2 is an immunotherapeutic target in human malignant glioma (55).

DCT-mediated pathway of tumor cell resistance to environmental and therapeutic stress. The most recent information about new biological functions of DCT in pathological state indicate that DCT mediates a pathway related to the response to environmental and therapeutic stress in cells of melanocytic lineage. Investigations about melanocyte or melanoma resistance to environmental stress showed that TRP-2 protects these cells from UV-or X ray-induced apoptosis (56, 57). A common characteristic in both melanoma and glioma tumor cells is their intrinsic and acquired resistance to chemotherapeutic drugs. In an attempt to identify the genes that confer resistance to cis-diaminedichloroplatinum (II) (CDDP), using the strategy of retroviral insertion mutagenesis, Lu *et al*. selected clones which had 2–3 fold increase in level of CDDP resistance relative to parental WM35 melanoma cells (58). The analysis of WM35 CDDP-resistant clones (59) showed an overexpression of TRP-2, suggesting its involvement in the elevated resistance to CDDP of these cells. Moreover, enforced expression of TRP-2 in WM35 parental cells by transfection elevated their resistance to CDDP and this process was accompanied of the reduction in CDDP-induced apoptosis. Human melanoma DCT-mediated resistance to CDDP was shown to be independent of TRP-1 and Tyr expression (60). Interestingly, the melanoma CDDP-resistant clones have also shown resistance to methotrexate and carpoplatin, but not to taxol. All together, these studies demostrated that, in tumor cells, DCT specifically controls a pathway related to resistance induced by chemotherapeutic alkylating agents and radiation and suggested that the mechanisms by which DCT mediates this process could be related to its enzymatic activity (61). Indeed, very recent studies provided evidence that DCT overexpression in melanoma cells reduced their sensitivity to oxidative stress, whereas following silencing DCT expression by RNA interference these effects were specifically reversed. Furthermore, these properties depended on a particular cell environment since cells of nonmelanocytic lineage stably transfected with DCT failed to be rescued when were exposed to similar treatments (62). However, when HEK cells expressing DCT were exposed to dopamine and hydroquinone at toxic concentrations their sensitivity to both compounds was significantly reduced. This beneficial property of DCT was related to the integrity of the DOPAchrome tautomerase catalytic site. The model proposed by the authors by which DCT mediates a detoxification pathway in nonmelanocytic

cells is related to the shared homology between quinones derivatives with DCT natural substrate L-DOPAchrome, and to the fact that DCT may have a possible oxidoreductase activity. Alternatively DCT would support a privileged pathway in which GSH pool is preserved by diminishing intermolecular single–electron exchange that produces highly reactive semiquinone derivatives (63).

DCT as a possible new molecular marker in the diagnosis of skin lesions. A particularity of neoplasm cells derived from melanocytes, which enable these tumor cells to escape immune recognition, is downregulation of melanosomal antigens (64). This event also represents a drawback in melanoma diagnosis and staging. The morphologic diversity of melanoma often requires the diagnosis based on immunohistochemical staining. Despite the numerous molecular markers used by different laboratories, there is still no consensus strategy for the optimal detection of melanoma by immunohistochemistry. Several studies show that, unlike other melanosomal proteins, DCT expression is retained in many unpigmented melanomas (65, 66) and recommend DCT as a specific and sensitive antigen for diagnosis of amelanotic melanoma (67). However, no consistent evaluation of human melanocytic lesions using DCT as molecular marker has been conducted so far.

CONCLUDING REMARKS – FUTURE PERSPECTIVES

After more than one decade since DCT gene has been cloned and sequenced, DCT is no longer viewed only as an enzyme that controls a secondary step in melanogenetic pathway.

DCT structural characteristics and the molecular mechanisms which specifically regulate its cellular expression are distinct from the other two homologous TRPs and support the idea that DCT may accomplish additional functions in the cell. The identification of DCT sequences (epitopes) representing targets of cellular and umoral response in melanoma/glioma or vitiligo urged the development of novel approaches which combine DCT-based vaccines with adjuvant-(immuno) therapy. A new pathway which reduces tumor cell sensitivity to environmental and chemo-/radio-therapeutic stress mediated by DCT has been demonstrated. This represents a challenge for scientists to understand novel intrinsic and acquired mechanisms of melanoma/glioma resistance and to design new therapeutic strategies. The DCT participation in the regulation of neural progenitor cell proliferation and neuroblast migration suggests important but yet insufficiently explored functions of DCT in the nervous system.

Despite the large body of information about DCT, there are still some unanswered questions: What is the mechanism by which TRP-2 prevents quinone toxicity? What other proteins are involved in DCT-mediated tumor resistance? Could be DCT involved in melanoma cell migration? Could DCT represent a prognosis

marker in melanoma development and thus an indicative for the selection of therapy with specific pharmacologic agents? Further studies will be needed to answer these questions which would link DCT to many challenging topics.

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