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TRANSFUSION CLINIQUE ET BIOLOGIQUE

Transfusion Clinique et Biologique 17 (2010) 184–196

Malaria

Multiple interests in structural models of DARC transmembrane protein

Multiples intérêts des modèles structuraux de la protéine transmembranaire Darc

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Available online 23 July 2010

Abstract

Duffy Antigen Receptor for Chemokines (DARC) is an unusual transmembrane chemokine receptor which (i) binds the two main chemokine families and (ii) does not transduct any signal as it lacks the DRY consensus sequence. It is considered as silent chemokine receptor, a tank useful for chemiotactism. DARC had been particularly studied as a major actor of malaria infection by *Plasmodium vivax*. It is also implicated in multiple chemokine inflammation, inflammatory diseases, in cancer and might play a role in HIV infection and AIDS. In this review, we focus on the interest to build structural model of DARC to understand more precisely its abilities to bind its physiological ligand CXCL8 and its malaria ligand. We also present innovative development on VHHs able to bind DARC protein. We underline difficulties and limitations of such bioinformatics approaches and highlight the crucial importance of biological data to conduct these kinds of researches.

Keywords: Transmembrane protein; Comparative modelling; Bioinformatics; Protein docking; Flexible docking; Camelid VHHs; Malaria; Cancer

Résumé

Le *Duffy antigen receptor for chemokines* (DARC) est un récepteur aux chimiokines inhabituel qui (i) lie les deux grandes familles de chimiokines et (ii) du fait de l'absence du motif DRY ne transduit pas de signal. Récepteur silencieux, il est un réservoir utile pour le chimiotactisme. DARC a été particulièrement étudié comme un acteur majeur de l'infection par *Plasmodium vivax*. Il est également impliqué dans des maladies inflammatoires, cancers et pourrait jouer un rôle dans l'infection par le HIV. Nous présentons l'intérêt de construire un modèle structural de DARC, pour comprendre plus précisément sa capacité à lier son ligand physiologique CXCL8 et son ligand paludique. Nous présentons des développements innovants portant sur des VHHs capables de lier le DARC. Nous soulignons aussi les difficultés et les limites des approches bioinformatiques et mettons en évidence l'importance cruciale de données biologiques pour mener à bien ce type de recherches.

Mots clés : Protéine transmembranaire ; Modélisation comparative ; Bioinformatique ; Assemblage protéique ; Assemblage flexible ; VHHs de camélidés ; Paludisme ; Cancer

1. DARC

The history of human knowledge on Duffy Antigen Receptor for Chemokines (DARC) begins in 1950 with the discovery of a new blood groups system (the Duffy blood group system) named from the person who developed the first antibody against the so-called Fya antigen [1]. A second antithetic antigen Fyb [2] was shortly after discovered. In 1955, it was shown that antigens of Duffy blood group system were missing in red blood cells (named Fy(a-b-)) from a large proportion of West African ascent population (RBC-WAAP) [3]. It was observed thereafter that these cells were resistant to

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1246-7820/$ – see front matter
doi:10.1016/j.tracli.2010.05.003

invasion by *Plasmodium vivax* (see below). Other important steps were cloning of cDNA coding for the protein carrying the Fy antigens, the Duffy glycoprotein, the recognition that Duffy glycoprotein was a transmembrane receptor for chemokines leading to changing its name to DARC.

In this short review, we will briefly overview knowledge on the immunogenic properties of DARC, relations of DARC with malaria, of DARC with chemokines inflammation and inflammatory diseases. We will quote present research which deals with the multiple roles of this somewhat enigmatic protein that, besides malaria and inflammation, is implicated in cancer and might play a role in HIV infection and AIDS. Then, we show the interests in the design of structural models for DARC analysis. We will present (i) how to build proper structural models of DARC [4], (ii) how to elucidate pertinent interactions with its ligands [5] and (iii) what might be the role of structural modelling in elaboration of new tools for DARC studies [6].

1.1. Duffy antigens

They have been defined by studying reactivity of patients immunized through transfusion or pregnancy. Fya/Fyb allotypic variants exist and correspond to a SNP in exon 2 encoding a Gly42Asp substitution in the extracellular N-terminal domain of the Duffy glycoprotein [7,8]. Two other antigens have been identified: (i) Fy3, which involves residues from the third extracellular loop [9], is probably a conformational reader and (ii) Fy6, which was discovered after immunisation of mice with human red cells or engineered eukaryotic cells expressing DARC, is a linear epitope contained in the first extracellular domain. Fy6 is present both in Fya or Fyb allotype, and only Fy(a-b-) cells do not react with anti Fy6.

The mechanism of selective extinction of expression of Duffy related antigens on WAAP red cells have been elucidated. The Duffy negative phenotype of WAAP red cells (noted Fy(a-b-)) is due to homozygosity for a promoter polymorphism (-46C) in which the binding site for the transcription factor (GATA-1), required for DARC to be expressed on the cell surface of erythrocytes [10], is disrupted. This mutation is present in a Fyb genetic background. Importantly, in Fy(a-b-) WAAP, DARC is normally expressed on cells in which DARC expression was already demonstrated for example, endothelial cells of postcapillary veinules, epithelial cells of collecting ducts of the kidney, cerebellar Purkinje cells [11,12]. Another promoter is likely operative in these tissues.

1.1.1. DARC and Plasmodium vivax

DARC was characterized as an erythrocyte receptor for malaria parasite through *in vitro* studies and also *in vivo* experiments performed on American volunteering detainees [13,14]. The hypothesis that DARC might be a receptor for *P. vivax* raised after it was noted that WAAP might be resistant to infection by *P. vivax* purportedly performed to treat neurosyphilis. All this does support the widely accepted hypothesis that *P. vivax* was the driving force for fixing the mutation silencing red cell expression of DARC. In this regard, it is interesting to note that in Papua New Guinea, where *P. vivax* malaria is also endemic, heterozygous individuals for the same GATA-1 site mutation have been found [15] but on a Fya background. It is tempting to speculate that the same FY GATA-1 mutation in Africans and Melanesians occurred independently in these two populations as a result of the same selection pressure.

Plasmodium vivax Duffy binding protein (PvDBP) is a merozoite microneme ligand vital for blood-stage infection, which makes it an important candidate vaccine for antibody-mediated immunity against *vivax* malaria [16,17]. Naturally acquired antibodies to DBP seem to confer protection from blood-stage *P. vivax* infection, supporting the development of a vaccine against *P. vivax* malaria [18]. However, other studies also pinpointed that produced human antibodies might have low efficiencies underlining the difficulty of vaccine design [19]. Hence, alternative approaches to interfere with *P. vivax* merozoite with DARC on red cells are demanded. Consequently, analysis of interaction mechanisms between DARC and DBP is important; analysis of DBP variants and DARC genotypes gives also insights to the sequence–function relationship [20].

Very recently, studies have shown that in Madagascar, *P. vivax* can invade Fy(a-b-) erythrocytes leading to disease [21]. Further studies are necessary to identify the genetic peculiarities of the parasite strain, the receptors that enable this DARC-independent *P. vivax* invasion of human erythrocytes.

Beyond DARC and PvDBP, it is worth to notice the existence of Duffy-binding like (DBL) domains implicated in other types of malaria. Domains related to PvDBP are found in *Plasmodium falciparum*. DBL domains are conserved regions of erythrocyte membrane protein 1 (PfEMP1) family. VAR2CSA Duffy binding-like (DBL) domains, which bind chondroitin sulphate A in placenta, are interesting candidates for the development of a vaccine against pregnancy-associated malaria [22]. Indeed, in spite of the extreme polymorphism of PfEMP1 DBLα domains, specific antibodies reducing risk of malaria in areas with high transmission rates were acquired [23]. DBLs family fold is supposed to be conserved. Consequently, the family is intensively studied to elucidate binding mechanisms [24–27].

1.1.2. DARC and chemokines

DARC is a transmembrane receptor for a variety of chemokines of both CXC and CC classes, including angiogenic (ELR^+) CXC chemokines, but not angiostatic (ELR^-) CXC chemokines [28,29]. DARC sequence is quite different from other chemokines receptors [30,31]. It is a silent chemokine receptors (or interceptors) [29]. Besides, a clear distinction should be made between DARC expressed on red cells and DARC expressed in other tissues. Importantly, DARC is lacking the DRY consensus sequence that is necessary to activate a protein G dependent activation cascade after activation by ligand binding [8,32–34]. DARC on red cells does not internalize. DARC might play the role of a buffer or a scavenger for chemokines and could reduce their concentration