stages. Since folate metabolism would still be essential even under slow-growing conditions, malaria parasites must salvage enough folate-related compounds either from the blood meal or from the mosquito host. In either case, a lower demand for *p*ABA/folates in the mosquito stages would make it harder to interfere with folate metabolism to block malaria transmission. Similarly, the ADCS KO parasites were able to complete liver-stage development normally and cause blood-stage infections in mice without extra *p*ABA supplementation. This is likely due to a rich supply of folates concentrated in animal livers, which renders active *p*ABA *de novo* synthesis nonessential in the parasites.

The major conclusion from this study [1] is that *Plasmodium* parasites use a combination of salvage and synthesis to ensure an adequate supply of *p*ABA (and folates) to support their fast growth in the asexual blood stages. Yet, a few key questions remain to be answered. Does *p*ABA synthesis fluctuate according to the quantity of *p*ABA or related precursors available for salvage? In animal models, can we develop methods to completely restrict *p*ABA salvage sources from diets and the host's microbiota? If *p*ABA salvage is indeed the main supply route over the entire life cycle, why have the enzymes of the *p*ABA *de novo* synthesis pathway not been abandoned during evolution as an unnecessary waste of resources, as they have been in mammals?

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https://doi.org/10.1016/j.pt.2019.01.010

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Forum

Fighting Cancer Using an Oncofetal Glycosaminoglycan-Binding Protein from Malaria Parasites

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Malaria research has led to the discovery of oncofetal chondroitin sulfate, which appears to be shared between placental trophoblasts and cancer cells and can be detected by the evolutionary refined malaria protein VAR2CSA. Interestingly, using recombinant VAR2CSA to target oncofetal chondroitin sulfate shows promise for novel cancer diagnostics and therapeutics.

The Oncofetal Hypothesis: Trophoblasts and Cancer Cells

Cancer is a leading cause of morbidity and mortality. The disease covers a group of more than a hundred different cancer types all sharing the feature of abnormal and uncontrolled cell growth [1]. Strategies for cancer diagnostics and therapeutics are often based on targeting nonexclusive but overexpressed markers in tumor tissue or body fluids, which limits the sensitivity and specificity for diagnostics and leads to various severe side effects during cancer treatment. In the quest of improving cancer outcomes, decades of research have focused on the identification of a universal but specific magic bullet to target all cancers.

The search for an omnipresent cancer target has included oncofetal markers. Such markers will be present during fetal or placental development but reduced to nondetectable levels in matured tissue. The expression of an ideal oncofetal marker reappears in a broad repertoire of tumor tissue, whereas it remains dormant in nonmalignant tissue. The search for oncofetal targets dates back to 1902, when the embryologist John Beard first proposed the trophoblastic theory of cancer (Box 1) [2]. Even though Beard considered cancer to arise from the germ cells, he described the cancer cells as similar to trophoblasts. In this regard, special attention has been drawn to the placenta. The majority of the cells forming the placenta are trophoblasts. During early embryonic development, these cells rapidly multiply and create their own blood supply. Furthermore, they invade the surrounding maternal tissue and resist immune surveillance. Interestingly, all of these trophoblastic characteristics are also essential traits of cancer cells. Regardless of whether Beard's hypothesis conforms to reality, the phenotypic comparison of cancer cells with trophoblasts remains intriguing.

Box 1. The Trophoblastic Theory of Cancer

The search for cancer markers has led to an interest in oncofetal markers. This quest historically started with the oncofetal trophoblast hypothesis, stating the phenotypic resemblance of cancer cells to placental trophoblasts. The embryologist John Beard first proposed the trophoblastic theory of cancer in 1902. During embryonic development, primordial germ cells migrate to the genital ridge where they give rise to spermatozoa and oocytes. Beard suggested that a subset of germ cells lost their way during embryonic development and thus resided in other tissues, where they could initiate the development of tumors later in life. Beard described the cancer cells as similar to trophoblasts depite their germ cell origin. He hypothesized that this is caused by the microenvironment of the foreign tissue, in which the pluripotent germ cells would not be supported in their destined sexual differentiation but would instead undergo asexual division resulting in an outgrowth of cells similar in structure and nature to trophoblasts. These cells would further be able to differentiate in the direction of the adjacent normal tissue and thus adapt to the surrounding environment. The normal function of placental trophoblasts is to sustain a high growth rate by facilitating vascularization as well as invading the maternal uterus while still avoiding immune surveillance. Interestingly, these are features are shared with cancer cells.

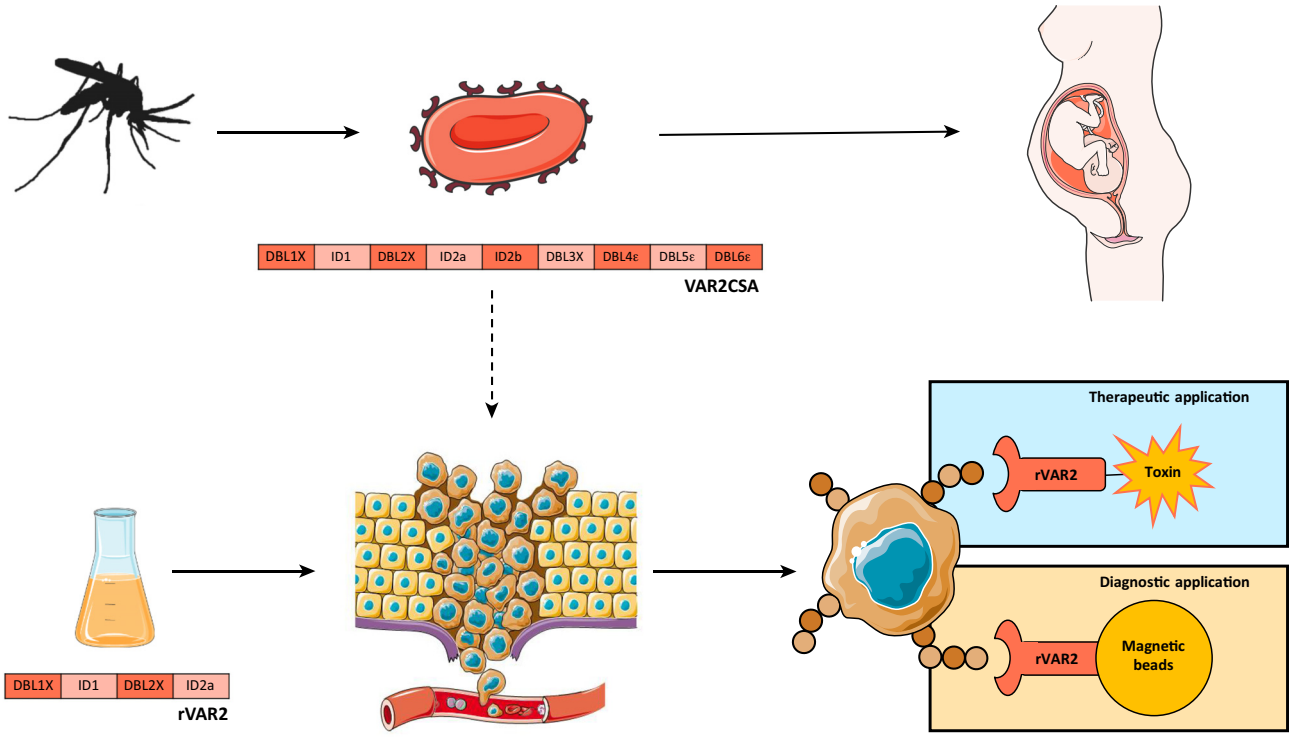

Figure 1. Using the Malaria Protein VAR2CSA for Cancer Therapeutic and Diagnostic Purposes. During pregnancy-associated malaria, the malaria parasites express a protein called VAR2CSA, which is displayed on the surface of the infected erythrocytes. VAR2CSA enables specific anchoring of the erythrocytes to the syncytiotrophoblast in the placenta by binding to oncofetal chondroitin sulfate (ofCS). Intriguingly, ofCS is also expressed by tumors, and VARCSA-expressing parasites or recombinant VAR2CSA (rVAR2) can specifically bind to a wide range of cancer cell lines and tissues of hematopoietic, epithelial, and mesenchymal origin. This can, for example, be exploited for cancer therapeutics by conjugating a toxin to rVAR2, or for cancer diagnostics by using rVAR2-coupled magnetic beads to capture circulating tumor cells in a blood sample. This figure was created using templates from Servier Medical Art website (https://smart.servier.com/).

Placental Malaria and VAR2CSA

Based on our previous work on developing malaria vaccines [3], we hypothesized that malaria could potentially be an important key for finding the long-sought oncofetal marker. It has been demonstrated that malaria-infected erythrocytes in pregnant women adhere in the placenta [4]. More specifically, the *Plasmodium falciparum* protein VAR2CSA has evolved to facilitate a specific and high-affinity binding of the infected erythrocytes to the placental syncytiotrophoblast [5].

During a malaria infection, the parasites infect the erythrocytes. This is a near-optimal habitat for the parasite as it requires hemoglobin and hides from the immune system due to the lack of MHC molecules on erythrocytes. However, as the parasites grow and divide inside the

erythrocytes the cells become swollen and will be recognized as abnormal and cleared by the spleen. To avoid the circulation, and thereby splenic clearance, the parasites express proteins on the surface of the infected erythrocytes that effectively anchor them to the vascular endothelial lining. The parasites have a repertoire of these proteins, all belonging to the PfEMP1 protein family, but with different receptor specificities. Fortunately, over time, an individual being exposed to several malaria infections acquires a large repertoire of PfEMP1 variant specific antibodies enabling protection and control over infections with *P. falciparum*. However, in a pregnant woman, the appearance of the placenta creates a new niche for the infected erythrocytes. By expressing the VAR2CSA protein, a variant not encountered previously by the human host, the parasite can once again adhere and accumulate in the host, causing inflammation and inhibition of blood flow to the fetus.

During pregnancy-associated malaria, VAR2CSA binds to a specific type of glycosaminoglycan comprised mainly of chondroitin sulfate A (CSA) [5,6]. Malaria parasites isolated from the placenta exclusively upregulate the *var2csa* gene, and disruption of the *var2csa* gene has been shown to cause loss of binding to CSA and the placental syncytiotrophoblast. Furthermore, expression of VAR2CSA is diminishable in non-CSA-binding malaria isolates. Finally, naturally acquired immunity to pregnancy-associated malaria, which is acquired as a function of parity, is correlated with elevated levels of VAR2CSA-specific IgG. The exclusive CSA-dependent placental sequestration of VAR2CSA-expressing erythrocytes highlights the existence of a unique type of placental CSA distinct from that expressed by any other organs. Intriguingly, this specific chondroitin sulfate (CS) turned out to be not only of interest to researchers trying to develop protective vaccines against pregnancy-associated malaria, it also became the path to discover a novel oncofetal marker.

Cancer and CS

CS is not restricted to the placental tissue but is found throughout the various tissues of the body. The structures of glycosaminoglycans are, however, extremely diverse as the assembly is not based on a precise synthesis template but a vast number of enzymes that are tightly regulated based on tissue and cell type. Furthermore, an increase in complexity is caused by the display of one or multiple CS side chains to a large family of proteins called proteoglycans. It is well known that cancer cells express aberrant chondroitin sulfate proteoglycan (CSPG) levels [7]. CSPGs are implicated in cancer through their direct role in cellular signaling. The CS side chains or the protein core can bind effector molecules and connect growth factor receptor complexes and extracellular matrix components to transmit pro-oncogenic signaling events. Although the majority of cancers seem to have an upregulation of some CSPGs, the variability of CSPGs within different tumor tissues is striking. Due to the great diversity among the protein cores of the cancer-associated CSPGs, it is likely that a common cancer feature is not found in the protein cores but rather as a specific CS modification. Unfortunately, characterization and targeting of CS in cancers have been a technical challenge due to low specificity of conventional typing reagents towards the poorly defined and complex glycosaminoglycan structures.

rVAR2 Targeting Cancer

Based on the hypothesis that cancer cells re-express the placental type of CS, thereby conferring an invasive and rapid growing cellular phenotype, we showed that infected erythrocytes expressing VAR2CSA adhere to a wide range of different cancer cell lines *in vitro* (Figure 1) [8]. The specific CS structure, which seems to be shared between trophoblasts and cancer cells, was termed oncofetal CS (ofCS). Following this observation, the binding of recombinant VAR2CSA (rVAR2) to a wide array of cancer cell lines and human cancer tissues was confirmed. In summary, 95% of patient-derived human cancer tissues and cell lines of hematopoietic, epithelial, and mesenchymal origin were shown to be ofCS-positive, while non-malignant tissue and cells displayed low-to-absent binding of rVAR2. ofCS is present on several well-known cancer-associated proteoglycans, suggesting an essential role of ofCS in oncogenesis, and possibly explaining the omnipresence of rVAR2 binding across a wide panel of cancers. Current research is exploring the diverse applications of rVAR2 in cancer diagnostics and therapeutics. In *in vivo* xenograft animal models, it has been demonstrated that intravenous injection of rVAR2 locates to the tumor and that toxin-conjugated rVAR2 effectively halts tumor growth in diverse cancer models. Recently it was demonstrated that rVAR2 could be used to capture circulating tumor cells [9]. This is in line with data showing that ofCS is involved in cellular migration and metastasis formation [10]. Although the malaria VAR2CSA protein up to now is the only pathogenic antigen that binds exclusively and with high affinity to ofCS, multiple other pathogens, including parasites, bacteria, and viruses, infect the human host by binding to glycosaminoglycans. Indeed, Kines *et al*. recently demonstrated that the glycosaminoglycan-binding properties of human papillomavirus can be used to specifically target uveal melanoma, and clinical trials have been initiated [11,12]. Perhaps, and hopefully, these will not be the only cases where seemingly pathogenic, but evolutionarily refined, organisms turn out to be helpful in fighting human disease.

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https://doi.org/10.1016/j.pt.2018.11.004

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