Metadichol® and CD33 Expression in Umbilical Cord Cells

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Abstract

CD33 also known as Siglec-3 is endogenously expressed in stem cells and is a marker for the myeloid lineage of cells. Increased expression of CD33 thus allows it to bind to any Sialic Acids (SIAs). These acids are binding sites for pathogens and toxins. By binding to these acids, CD33 can prevent invasion of hosts by these pathogens. Down-regulation of CD33, increase the release of the pro-inflammatory cytokine TNF-α by monocytes that increases reactive oxygen species that are involved in diseases like diabetes mellitus, Alzheimer’s, cardiovascular diseases asthma, and in various cancers.

The up-regulation of CD33 using Metadichol® was studied using Wharton’s Jelly Mesenchymal Stem Cells (MSCs) isolated from human umbilical cord and were grown in p-35 dishes until confluent and carried treatment was carried out with different concentrations. One dish was untreated and considered as control. The treated and untreated cells were analyzed using Flow Cytometry. The cells treated at 100 pg of Metadichol® has shown the highest increase (>400 fold) in CD33++ expression (48.77%) compared to untreated control (0.11%).

Keywords: CD33; Cord blood; Stem cells; Multipotency; Umbilical cord blood; Metadichol®; AHR; VDR; Inverse agonist; TNF-α; Cancer; Diabetes

Abbreviations: MSCs: Mesenchymal Stem Cells; SIA: Sialic Acid; HSCs: Hematopoietic Stem Cells; AML: Acute Myeloid Leukemia; GO: Ozogamicin; VDR: Vitamin D Receptor; UCB: Umbilical Cord Blood

Introduction

CD33 is a transmembrane protein of the Siglec family [1]. Siglecs bind to SIAs and are expressed by Hematopoietic Stem Cells (HSCs) which are present in Umbilical Cord Blood (UCB) cells. Humans express a larger family of CD33 related siglecs. Innate response is the body’s first line of defense, but it can also result in damage to host over time. Siglecs act as a negative regulator in immune cells like monocytes and macrophages to minimize damage [2]. This interaction is essential in balancing the signals in a cell to bring about homeostasis. The best-known Siglecs are CD22 and CD33. Over the last two decades, many more Siglecs have been identified. Siglecs play a role in the regulation of immune systems by binding to SIAs. Humans express a larger family of CD33 related siglecs and can be present on the same cells, e.g., monocytes express siglec-3, 5, 7, 9 and 10. Siglecs can carry out immunological functions that could be positive or negative. For e.g., creating a barrier against pathogens thus reducing interactions and at the same time weakening complement activation [3,4].

CD33 is a critical molecule in the inflammatory response, depending on the SIA microenvironment for its repressor activity. Reduced CD33 leads to an increase in inflammatory cytokines, such as IL-1β, TNF-α, and IL-8. Hyperglycemia down-regulates CD33 expression and triggers the spontaneous secretion of TNF-α by peripheral monocytes and increased free radical generation. CD33 thus has a vital role in the pathology of with type 2 diabetes [5,6]. SIA occupies the terminal end of cell glycan molecules on the surfaces of cells. Pathogenic bacteria have evolved to cover themselves in SIA that allows them to circumvent the host silica acid taken up by pathogens

Table 1: Examples of pathogens that bind to sialic acids on human cell surfaces.

| Pathogens                          | Diseases                        |
|-----------------------------------|---------------------------------|
| Neisseria meningitides B          | Meningitis                      |
| Escherichia coli K1               | Neonatal Meningitis             |
| Group B Streptococcus             | Neumonal and infant infections  |
| Campylobacter jejuni              | Enteritis, Guillain-Barre syndrome |
| Host silica acid taken up by pathogens |  |
| Hemophilus influenzae             | Respiratory infections          |
| Hemophilus ducreyi                | Chancroid                       |
| Sialic acid transferred by trans-sialidase |  |
| Trypanosoma Cruzi                 | Chagas disease                  |
| Carinabacterium diphtheriae       | Diphtheria                      |

Table 2: List of pathogens that release sialic acid and diseases associated with them.

sialidase-expressing bacteria living in the same niche [8] or by the host in the course of inflammation [9-12].

Treatment of human monocytes with anti-CD33 mAb induces the production of the pro-inflammatory cytokines IL-1β, TNF-α, and IL-8. CD33 ligand removal from the monocyte surface by neuraminidase...
resulted in IL-1b up-regulation, controlling monocyte activation. CD33 at the cell surface is crucial to maintaining monocytes in the resting state. If there is decreased, levels of SIA in the microenvironment CD33 will induce IL-1b production by monocytes, thus participating in the delivery of alarm signals [13].

CD33 is found to be expressed in 90% of Acute Myeloid Leukemia (AML) blasts [14]. Clinical data have been shown with immunoconjugates consisting of the humanized anti-CD33 antibody (gemtuzumab) and the cytotoxic drug calicheamicin (ozogamicin) as an effective anti-AML regimen. Ozogamicin (GO), the first drug conjugate approved for clinical use for anti-leukemia therapy [15]. After binding to CD33-antigens, gemtuzumab is taken into the cell via endocytosis. A new expression of CD33-antigens becomes necessary on the cellular surface of myeloid cells leads to endocytosis of gemtuzumab present, leading to efficacy in killing cells [16].

The low expression of CD33 and the slow internalization of CD33/antibody complexes leads to relatively limited CD33-mediated drug uptake per unit of time; consequently, for an anti-CD33 antibody-drug conjugate to be most successful, a highly potent toxin will be required [17]. Several novel CD33-targeted therapeutics that may overcome some of the limitations of earlier therapeutics are currently in preclinical and early clinical development. The low expression of CD33 and the slow internalization of CD33/antibody complexes leads to relatively limited CD33-mediated drug uptake [18]. Targeting CD33 proved to be difficult and GO withdrawn from the U.S. and European markets because of a lack of overall efficacy [19].

We recently showed that UCB cells on ex vivo treatment with Metadichol a Nanoemulsion of long-chain alcohols showed enriched CD34+ cells [20]. Metadichol is an inverse agonist [21] of Vitamin D Receptor (VDR), a TNF-α inhibitor [22] and anti-diabetic agent as well as anti-microbial agent [23,24]. This paper deals with Metadichol expression on UCB cells leading to increased CD33 expression similar to what we saw in CD34 expression. The increased expression observed with CD33 could be one possible mechanism that could explain its diverse pharmacological actions of Metadichol on multiple diseases [25-38].

Experimental

Work was outsourced and carried out by Skandia Labs, Pvt Ltd, Bangalore India Antibody: CD33-FITC Conjugate purchased from BD Pharmingen.

Cell line: Umbilical Cord Blood (UCB) cells,

Procedure:
1. Culture 1 × 10^6 cells in a 6 well plate containing 2 ml of complete media
2. After 24 hrs of incubation, cells are treated with 1 pg, 100 pg, 1 ng, 100 ng and 1 µg in serum-free DMEM media and incubated for 72 hrs
3. After 72 hrs of treatment, cells were collected and, pelleted cells at 4000 rpm for 5 minutes at room temperature and discard the supernatant

Figure 1: Results of UCB cells treated with Metadichol®.
4. Wash the cell pellet twice with 1X PBS
5. The cell pellet was re-suspended in 100 µL Sheath fluid and incubated with CD33-FITC antibody for 20 minutes in the dark
6. Post incubation, the cells were once washed with 1X PBS and resuspended in Sheath fluid
7. The treated and untreated cell populations were determined using FACS Caliber (BD Biosciences, San Jose, CA)

Figure 1 shows the results of UCB cells treated with Metadichol® 1 pg, 10 ng, 100 ng and 1 µg of Metadichol® for 72 hrs. The cells showed ten (CD33+) to hundred (CD33++) fold increased expression of CD33. Cells treated at 1 ng Metadichol® showed a 15 fold increase in expression of CD33+ cells (77.52%) compared to control cells (5%). The cells treated at 100 pg showed highest increase (>400 fold) in CD33++ expression (48.77%) compared to untreated control (0.11%). The reason we believe that there is less expression at higher concentration may have to do with the pH which about 4.5 and this can affect some but not all cell lines. In virus cell lines, we have noticed that it is toxic to cancer cells at higher concentrations.

Discussion

We have shown previously that Metadichol® binds to VDR whose natural ligand is Calcitriol. We have published Metadichol’s action on biomarkers implicated in various diseases [25-28]. It exhibits antimicrobial activity against parasites, viruses, and bacteria. The shortest interaction branch, between the two genes VDR and CD33 is shown in Figure 2. It was generated by a web-based program ConsensusPathDB [39]. CD14 is an endogenous ligand for CD33 [40]. Calcitriol regulates CD14 in human cells. CD14 binding to CD33 leads to TLR4-mediated signaling plays an essential role in initiating the innate immune response [41].

Gonzales, et al., [5] have shown that diabetic patients have low levels of CD33 when compared to healthy control, where as the TNF-α levels are high. This work correlates with our studies that show Metadichol® reduces sugar levels and also TNF-α level in diabetic rats. All these downfield effects result from binding to nuclear receptor VDR (Figure 3).

Many pathogens incorporate SIAs into their surface glycoconjugates through various mechanisms [42]. Research supports the notion that SIA molecular mimicry can be exploited as a virulence to subvert host immune systems or to infect permissive target cells through an interaction with various Siglecs. Bacterial, fungus and viruses produce SIAs. Pathogenic microbes have evolved evolutionary mechanisms to interact with Siglecs. These pathogens displaying SIAs evade the immune system and thus dampen host immune response. SIAs are recognized and bind to CD33 related siglecs [43] and help maintain homeostasis of host innate immune response, allowing an inflammatory response that is activated upon sensing of danger-associated molecular patterns or pathogen-associated molecular patterns [44]. Enhanced expression of CD33 thus allows it to bind to any SIAs present and those released by pathogens and thus prevent infection of the host. Metadichol® is a food derivative and free of toxic effects [45-47]. It can be safely used alone or in combination with existing treatment protocols.

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