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Mini-review

SDA and IDA – Two aptamers to inhibit cancer cell adhesion

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ABSTRACT

Aptamers which bind to proteins involved in cell-cell interactions could have significant value to directly affect cancer cell adhesion or for directed cargo delivery. Here, I discuss two aptamers: aptamer SDA which binds to E- and P-selectin, and aptamer IDA which binds to α6β4 integrin. Both aptamers (SDA 91 nt and IDA 77 nt) bind their target proteins with dissociation constants in the 100–150 nM range and substantially inhibit special cellular adhesion, possibly a first and pivotal step in transendothelial migration during metastasis formation. The aptamers' half-lives in cell culture media are between two and six hours. IDA is internalized by integrin presenting cells within minutes thus possibly serving as vehicle for directed cargo delivery.

1. Introduction

This article reports exclusively about DNA aptamers – which are serious alternatives to antibodies with the sole difference that they are composed of nucleotides and not of amino acids. In recent decades, nearly each imaginable class of molecules, molecule complexes or even whole cells have served as targets for the selection of aptamers. One class of aptamers selected in our laboratory targeted different kinds of cell surface proteins like the human interleukin-6 receptor (hIL-6R). Neither the obtained DNA [1] nor RNA aptamers [2–4] did interfere with the human interleukin-6 (hIL-6) initiated signal transduction pathway. The RNA aptamer, however, was internalized by hIL-6R presenting cells and thus could serve as a vehicle for cargo delivery [2,5–7].

Another class of cell surface proteins which have been targeted with aptamers were proteins involved in cell-cell interactions. Such interactions are fundamental biological items and if they misbehave this might cause severe health problems culminating in cancer [8–10]. The selection of aptamers inhibiting cancer cell adhesion in general was recently reported in an excellent review by the Choulier laboratory [11]. Another attractive approach to tackle cancer with aptamers is to use them for constructing aptamer-siRNA chimeras, recently reviewed by Kruspe and Giangrande [12].

In our laboratory we also selected aptamers addressing particular cell surface proteins involved in cell-cell recognition or interactions, namely human selectins (**SDA**, [13]) and human and murine α6β4 integrin (**IDA**, [14]). Besides other reasons those mentioned targets were chosen as the corresponding aptamers might serve as inhibitors for the interaction of cancer cells with epithelial cells (Fig. 1) aiming at interfering with the adhesion of cancer cells. This is one of several necessary events that must take place to finally lead to the formation of metastases (Fig. 2; [15]). Only those two mentioned aptamers, SDA and IDA, should be dealt with in this short report.

2. SDA – a selectin specific DNA aptamer

2.1. Selectins

Selectins are long-stretched, carbohydrate-binding adhesion glycoproteins mainly present in the vascular system. Selectins have common domain structures and play a key role in the adhesion cascade of leukocytes. All three known selectins consist of a C-terminal calcium-dependent lectin domain (C-type lectin domain), an EGF (Epidermal Growth Factor)-like domain, and short consensus repeats (SCRs) followed by a transmembrane region and the C-terminal cytoplasmic region. They are divided into L- (leukocytes), E- (endothelium) and P- (platelet) selectins, depending on

Abbreviations: AA, amino acid; HPMEC, Human Pulmonary Microvascular Endothelial Cells; HT29, human colorectal cancer cells; IDA, integrin specific DNA aptamer; nt, nucleotide(s); SDA, selectin specific DNA aptamer; SELEX, systematic evolution of ligands by exponential enrichment; rhE- and rhP-selectin, recombinant soluble parts of human E- or P-selectin, respectively; sLe^A, sialyl Lewis A; sLe^X, sialyl Lewis X; TNFα, tumour necrosis factor alpha.

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0300-9084/

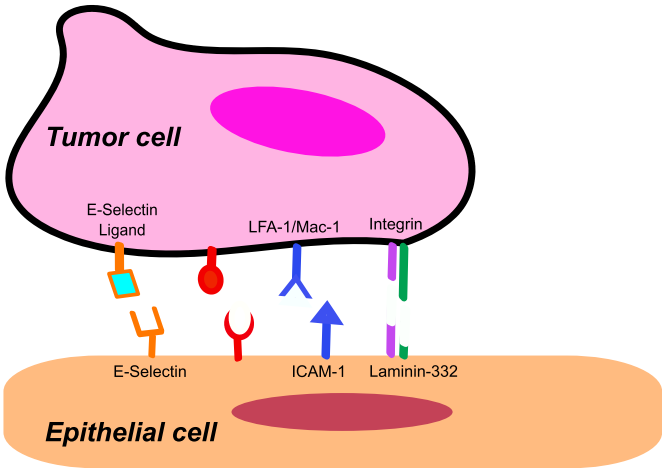

Fig. 1. Examples for surface protein mediated cell-cell interactions. This schematic drawing depicts a selection of cell surface proteins which might be involved in the interaction of cancer and epithelial cells. Two of those proteins, integrin and selectin, were chosen as targets for a DNA aptamer selection.

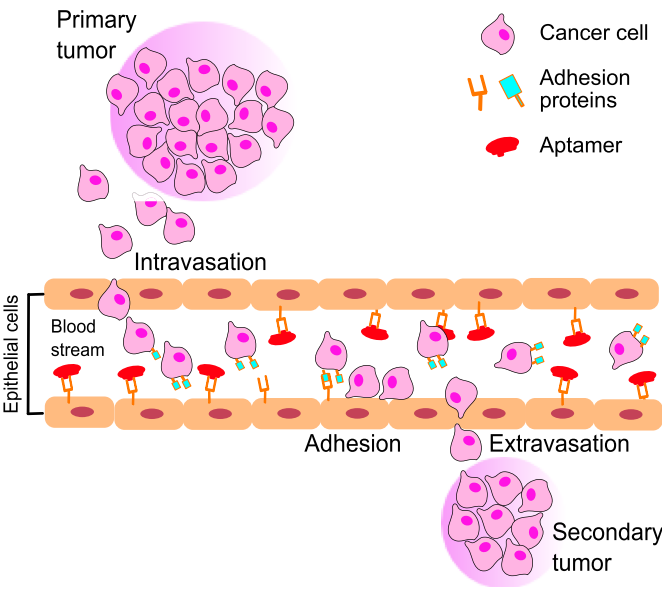

Fig. 2. Adhesion of cancer cells is the pivotal step in transendothelial migration during metastases formation. For the formation of metastases some prerequisites have to be fulfilled. After shedding of some cancer cells from the primary tumour these have to cross the wall of a vessel to enter the circulation where some of them, which are not endocytosed by phagocytes, might bind upon interaction of specific cell surface proteins to epithelial cells. This might result in an adhesion, which might be followed by an extravasation, finally leading to the development of a secondary tumour – a metastasis. This process might be interrupted at many possible stages [15] – one could be the masking of one of the involved cell surface proteins with a specific aptamer.

the cell type in which they occur [16,17].

Human P-selectin (hP-selectin) is the largest member of the selectin family with 830 amino acids (AAs) and a molecular weight of 140 kDa. The cytoplasmic region is formed of 35, the transmembrane region of 24, the C-type lectin domain of 101, and the EGF-like domain of 37 AAs. Nine SCRs, each containing 62 AAs, ensure the extension of P-selectin into the extracellular space. Twelve potential glycosylation sites and 23 disulfide bridges are described for hP-selectin.

Human E-selectin (hE-selectin) consists of 612 AAs and has a molecular weight of 115 kDa. 32 AAs form the cytoplasmic and 22 the transmembrane region, while 118 amino acids form the C-type lectin domain and 36 the EGF-like domain. In addition, hE-selectin consists of six SCRs, which, like the other selectins, consist of 62 AAs and have eleven putative glycosylation sites and 17 disulfide bridges.

The smallest member of the selectin family is the human L-selectin (hL-selectin) produced by leukocytes with only 372 AAs and a molecular weight of approximately 74 kDa. While, as in other selectins, the transmembrane region of the L-selectin consists of 23 and the EGF-like domain of 37 AAs, only 17 AAs form its cytoplasmic domain. The C-type lectin domain of the hL-selectin contains 101 AAs, as is the case for hP-selectin. With only two SCRs, the extracellular portion of hL-selectin is relatively small. This selectin has seven potential glycosylation sites and nine disulfide bridges.

In all selectins, the C-type lectin domain is responsible for binding the carbohydrate ligand. This interaction is dependent on the presence of calcium ions which leads to the affix “C-type” [18]. The AA sequences of C-type lectin domains show up to 65% sequence homology. This also explains the similar affinity of selectins for sialyl Lewis antigens [19]. In 1993 the corresponding ligand-binding epitopes for E- and P-selectin were described [20]. And also crystal structures were determined for both, E- [21] and P-selectin [22].

The selectins extend into the extracellular space with the SCRs, whereby the precise function of the SCRs has not yet been clarified. It is only known that P-selectin is no longer able to participate in lymphocyte homing when manipulating its SCR number [23]. The EGF-like domain, which separates the C-type lectin domain, responsible for the carbohydrate binding, from the SCRs, is attributed to a participation in the ligand binding of the selectins [24–27]. In addition, there are studies that make EGF-like domains and SCRs responsible for the ligand specificity of the selectins [28,29], although their exact role has not yet been defined. While the C-type lectin domain is highly conserved within the selectin family, no homology is present within the transmembrane and cytoplasmic regions of the selectins [30].

The crystal structures of the selectins show a globular C-type lectin domain with a flat ligand binding pocket lying at the surface. The selectins can always only interact with one ligand [21,22]. In all species the function of the selectins is identical, while the number of AAs, the SCRs as well as the number of glycosylation sites differ from species to species. For example, between human and murine L-selectins, there is an AA sequence homology of 77% with a 79% match in the nucleotide sequence [31].

While L-selectin is constitutively present on the surface of leukocytes, the presentation of E-selectin depends on the stimulation of the epithelial cells by cytokines such as TNFα and IL-1 or lipopolysaccharides (LPS) [32].

2.2. Selectin ligands

The selectin ligands are long-stretched, highly hydrophilic proteins which carry carbohydrate groups O-glycosidically linked at serine and threonine residues and a few carbohydrates N-glycosidically linked at asparagines. Since those ligands are fucosylated and sialylated mucin-like proteins they are also called sialomucins [33]. The carbohydrate-binding epitope of the selectins (C-type lectin domain) binds to the glycosidically linked sialyl-Lewis antigens of the sialomucins. Sialyl-Lewis-X (sLe^X) and its position isomer sialyl-Lewis-A (sLe^A) are the two most frequently sialylated and fucosylated oligosaccharides, which play a major role in selectin-mediated cell-cell recognition processes [33,34].

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