The expression and action of decay-accelerating factor (CD55) in human malignancies and cancer therapy

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Abstract. Decay-accelerating factor (DAF, CD55) is physiologically acting as an inhibitor of the complement system, but is also broadly expressed in malignant tumours. Here DAF seems to exert different functions beyond its immunological role such as e.g. promotion of tumorigenesis, decrease of complement mediated tumor cell lysis, autocrine loops for cell rescue and evasion of apoptosis, neoangiogenesis, invasiveness, cell motility, and metastasis via oncogenic tyrosine kinase pathways and specific seven-span transmembrane receptors (CD97) binding. Therefore, DAF has already become a target for therapy. In this paper we review the role of DAF in human malignancies as described in different basic, diagnostic and experimental therapeutic studies.

1. Introduction

The decay-accelerating factor (DAF), also designated CD55, was described for the first time in 1969 for its physiologic function within the human immune system when factors on the surface of erythrocytes were discovered regulating human complement activation [37,38]. The complement system as a part of the unspecific humoral immune system can take part directly in defence mechanisms as well as in control and regulation functions for the specific antibody mediated immune response. As uncontrolled activation of complement leads to damage of an organism and to excessive consumption of complement factors this system has to be regulated. This regulation is maintained in part by regulation proteins located in the cytoplasm and cell membrane. These complement regulating proteins include, besides DAF, C1-inhibitor, factor H, C4b-binding protein (C4bp), complement receptor 1 (CR1), Clusterin, Vitronectin, and CD59.

To date, DAF has been detected in all mammalians [35,54,85,87,91,107]. Physiologically it is expressed in all cells activating the complement system including peripheral blood, epithelial and endothelial cells [3, 50,73,82,83]. Moreover, soluble DAF is detectable in plasma, tears, saliva, and urine, as well as in synovial and cerebrospinal fluids [73].

The most well-known function of DAF is activation of the complement system by accelerating the decay of the C3/C5-convertase of the classic and alternative pathways of the complement system [13].

Besides its importance as a regulator of the complement system, DAF also seems to inhibit natural killer cells [24]. Moreover, DAF is also known as a receptor for certain viruses and other microorganisms [9,19,56, 92,101] and as a ligand of the CD97 receptor [29]. Excessive expression of different DAF isoforms in murine testicles suggests a further role of DAF in spermatozoa...
production and their survival within the female genital tract [32].

The clinical relevance of DAF comprises various diseases: paroxysmal nocturnal haemoglobinuria (PNH) [74, 98], autoimmune diseases such as rheumatoid arthritis, vitiligo, systemic sclerosis and psoriasis, as well as in some gastrointestinal diseases like ulcerative colitis [4, 30, 43, 99, 114, 116, 120]. In xenotransplantation DAF is used for the prevention of hyperacute and acute graft rejection [5, 21, 130].

Within recent years, expression of the decay-accelerating factor (DAF) has also increasingly been studied in human malignancies with regard to function and diagnostic and therapeutic strategies.

2. Structure and biosynthesis of DAF (CD55)

DAF is a glycoprotein appearing as different isoforms depending partially on different posttranslational glycosylation patterns [63] and sometimes on alternative splicing [15, 32, 35, 54, 87, 91]. Alternative splicing also determines the appearance of DAF molecules that are more hydrophobic or hydrophilic [15] and that vary in structure and localisation of the protein leading to glycosylphosphatidylinositol (GPI)-anchored membrane bound isoforms as well as to transmembranous and secreted variants [87]. On the mRNA level, two different DAF transcripts have been detected in human cells of 2.2 kb resp. 1.5 kb resulting from alternative splicing and possibly leading to a transmembranous as well as a secreted form of the protein [15].

Human DAF is preferentially expressed in cells exposed to the complement system such as hematopoietic and vascular endothelial cells [3, 50, 73, 82, 83]. The level of DAF expression seems to depend on the grade of maturation of normal hematopoietic cells [89]. DAF has also been detected in plasma, tears, saliva, urine, synovial and cerebrospinal fluids [73].

In different cell types a specific distribution pattern of DAF has been observed [10, 60, 133] suggesting an exact sorting mechanism for the transport of the molecule from the Golgi complex to the cell membrane [84]. Additionally, besides the membrane bound isoforms it is likely that in some human cells soluble and possibly secreted variants of this molecule occur [73, 80].

The gene encoding for DAF has been identified on the long arm of chromosome 1q32 within a locus encoding for different regulators of complement activation (RCA) [64]. These proteins, beginning at their amino terminus, consist largely of multiple copies of an approximately 60 amino acid short consensus repeat (SCR). The structure of the daf gene has already been extensively analysed [93]. It consists of about 40 kb and contains 11 exons. The length of these exons and introns varies considerably, with the exons ranging from 21 to 956 bp and the introns ranging from approximately 0.5 to 19.8 kb. SCR I, II, and IV are all encoded by single exons; however, SCR III is encoded by two separate exons, with the splice junction occurring after the second nucleotide of the codon for the glycine residue at position 34 of the consensus sequence. The structure of the DAF protein is also well known today (Fig. 1; [62, 65]). Every DAF molecule possesses four units for the control of complement activation following each other and that are designated as “complement control protein repeats” (CCP) 1 to 4. Every CCP consists of 60 amino acids and contains four cystein residues with always two of them being linked together [84] leading to a special folding of the CCPs [80]. Moreover, every DAF molecule disposes of a region that is rich in serine and threonine probably serving as a spacer to guarantee the required positions of the CCPs [13, 20]. Finally, most of the DAF isoforms are linked to the cell membrane by a glycosyl phosphatidylinositol (GPI) anchor following the four
CCPs, and this region is rich in serine and threonine [72].

Therefore, the molecular weight of the mature protein varies between 50 to 100 kDa in the different cell types which show DAF expression. Studies have shown that several smaller precursor forms are formed during the biosynthesis of DAF. Initially a precursor of 43 kDa is synthesized passing over to a 46 kDa precursor form by an early posttranslational modification. Finally, this transitional form is built up to the mature molecule [15,63,84].

3. DAF is frequently expressed in human malignancies

Like other complement inhibitors, DAF has been detected in different malignancies such as CLL, CML, ALL, AML, colorectal cancer, gastric cancer, thyroid cancer, medullary thyroid cancer, malignant glioma, breast cancer, renal cancer, non-small cell lung cancer, ovarian cancer, cervical cancer and in cell lines derived from those tumour types [10,27,31,44,51,66,78,79,86,105,118,129]. DAF expression has also been found in metastases of colorectal carcinomas [40]. Furthermore, studies have shown that DAF expression is frequently found within the stroma of colorectal tumors, which was explained by cleavage from the cell membrane of the tumour cells into the environment or secretion by the tumour cells as a soluble form [58,81].

Some tumors did not solely express a single variant of DAF but also expressed different isoforms of the protein (Fig. 2). Such isoforms can originate from different glycosylation patterns of DAF in some cell types like in some colorectal carcinoma cells [79]. In mammary carcinomas different isoforms of DAF were demonstrated to be due to alternative splicing [12].

4. DAF expression in malignancies is regulated by low molecular weight factors

Several factors leading to overexpression of DAF have been identified. IL-4, IL-1alpha and IL-1beta increase DAF expression in intestinal epithelial cells and human lung cancer cell lines [2,81,119]. The growth factor EGF induced DAF expression in colorectal cancer cells through a MAP-kinase dependent pathway [113]. Moreover, Prostaglandin E2 influenced expression of DAF in those tumors [39]. Some other cytokines like TNF-alpha and IFN-gamma can also stimulate DAF synthesis [71,108]. Thrombin as well as the growth factor bFGF increased DAF expression by a PKC dependent pathway [59,69]. Also PKC dependent induction of DAF synthesis by VEGF in endothelial cells [70] suggests a role of this protein in angiogenesis, the more so since DAF expression within quiescent endothelium is comparatively low [70]. This is further supported by the fact that cells of the breast cancer cell line MDA 231 were shown to increase DAF expression within tumor-associated endothelial cells by secretion of factors like VEGF [58]. Furthermore, CD59, another membrane complement regulatory protein, modulates DAF expression on human neuroblastoma cell surfaces [16].

5. DAF acts in malignancies as an immune modulator

Several functions have to be taken into consideration explaining the role of DAF overexpression in malignant tumours. The function the best known of DAF is its role as a complement inhibitor. Indeed, this function seems to be of high importance in melanomas, renal tumors, thyroid, lung, squamous cell and cervical carcinoma as well as in some haematological malignancies. In line with its physiological role in some cell types, increasing expression of DAF also decreases complement deposition on cell membranes as well as complement mediated lysis in these tumours [17,46,47,70,129]. It has also been shown that removal of DAF from the cell membrane or neutralisation of the protein increases the intensity of a local inflammatory reaction as well as complement mediated cell lysis, and could possibly also improve response to special therapeutic strategies [11,26,32,46,47,53,67,70,118,127,129,131]. This is further supported by the fact that DAF decreases cell adhesion of T-lymphocytes to U-937 human leukemic cells [49]. Therefore, DAF mediated decreased cell adhesion might be also play a role in invasive tumour growth and formation of metastases. DAF has also an inhibiting effect on natural killer cells which could promote tumour initiation and primary growth [24].

6. DAF induces tyrosine phosphorylation in signalling pathways

The GPI-anchored and membrane linked form of DAF is part of a signal transduction cascade leading
to tyrosine phosphorylation of several proteins. Specific phosphorylation occurred on tyrosine residues of p56lck, the TCR-zeta chain and ZAP-70 in CD3-positive Jurkat cells [103,115]. The molecules p56lck and p59fyn1 are tyrosine kinases of the non-receptor src-family [103,110,115]. Tyrosine kinases of the src-family and especially p56lck and p59fyn play also a decisive role in signal transduction in tumor cells [68,117]. Thereby, the tyrosine kinase p56lck participates in a signal cascade conveying active motility of breast cancer cells [68]. Further studies have shown that DAF dependent processes like those take also place in other cells like HeLa cervical carcinoma cells and neoplastic hematopoietic cells [90,103,104]. These processes can be inhibited by removal of the GPI-anchor [103].

As GPI-anchored molecules are localised at the external part of the cell membrane and not all protein tyrosine kinases possess an extracellular domain, it is supposed that further proteins participate here possibly building signal transducing complexes with DAF and the associated protein tyrosine kinases [18,52,103,109]. In any case, effects of DAF in cancer cells seem to enclose functions within signal transduction beyond its immune modulating effects.
7. **DAF is the ligand of the seven-span transmembrane receptor CD97**

DAF has been identified as ligand of the surface receptor CD97 [29]. CD97 (EGF-TM7) belongs to the family of class B seven-span transmembrane (TM7) receptors predominantly expressed by cells of the immune system. It was found that it plays an important role for the migration of neutrophil granulocytes [57]. CD97 is also ectopically expressed in human thyroid, colorectal, gastric, and pancreatic adenocarcinomas, and in esophageal and oral squamous cell carcinomas [7,8,36,77,78,111]. In thyroid and colorectal adenocarcinomas expression of CD97 correlates with advanced stage of the disease and increased dedifferentiation of tumor cells as well as with their potential for invasive growth, migration and formation of lymph node metastases [7,36,78,111]. Furthermore, CD97 stimulates angiogenesis [126] and in colorectal and gastric carcinomas it is preferentially expressed in cells at the invasive front of the tumors [8,111]. As its ligand DAF [28,29,128] is also synthesized and secreted by colorectal carcinoma cells [111], autocrine stimulation may occur. Indeed, in gastric and colorectal carcinomas, the expression of DAF was associated with invasion and metastasis [39,44,61]. A similar mechanism might also take place in breast cancer cells as c-erbB-2-positive mammary carcinoma cells showing increased transendothelial invasiveness selectively over-express and secrete a 45 kDa splice variant of DAF [12]. This splice variant might participate in invasive growth of these cells serving as an autocrine stimulus. Likewise, this smaller splice variant in comparison to the 70 kDa isoform of the protein might lack the GPI-anchor that is essential for signal transduction by DAF leading to a loss of inhibiting signals for migration and invasiveness of these cells.

The various actions of the DAF molecule in human malignancies are summarized in Fig. 3.
Table 1
Perspectives of DAF targeted therapies

| Targeted cancer                          | Therapeutic investigational design and benefit                                                                 | Reference |
|------------------------------------------|---------------------------------------------------------------------------------------------------------------|-----------|
| Stomach adenocarcinoma of diffuse-type   | Monoclonal antibody SC-1 against a gastric cancer specific 82 kD isoform of DAF; induced apoptosis in primary tumours as compared to pre-treatment biopsy material in up to 90% of the cases, regression of tumour mass up to 50% | [33,34,106,121,122,124,125] |
| Metastasizing gastric cancer in nude mice | Monoclonal antibody SC-1 against a gastric cancer specific 82 kD isoform of DAF; reduce the number of disseminated tumor cells in the bone marrow | [42]      |
| Non-Hodgkin’s lymphoma                   | Rituximab (chimeric anti-CD20 monoclonal antibody); enhancement of complement-dependent killing activity of Rituximab, by additional application of monoclonal anti-DAF-antibody | [132]     |
| Cervical cancer                          | Monoclonal antibody against DAF showed the widest range of specific reactivity | [41]      |
| Renal cancer                             | Bispecific antibodies binding DAF as well as renal tumour-associated antigen G250; decrease of unwanted side effects | [11,33]   |
| Osteosarcoma of children                 | DAF as a cancer vaccine additionally applied to myelo-suppressive chemotherapy; Induction of T-cell proliferation 71% and antigen-specific gammaIFN secretion in 59% of the cases; vaccination was well tolerated | [94]      |
| Melanoma xenografts in immunodeficient mice | Coxsackievirus A21 infection; rapid viral oncolysis                                                                 | [1,6,100,102] |

8. Future prospects: DAF in cancer diagnostics and as a therapeutic target

Based on the frequent expression of DAF in human malignancies it might be a useful protein for diagnostics. For instance, DAF is frequently detectable within the stool of patients with colorectal carcinomas and might therefore contribute to the early diagnosis of this disease [45,48,75,76,88]. Further studies have to be performed to reveal whether DAF will also obtain diagnostic relevance for other tumors, e.g. in immunohistochemistry or serum tumor marker diagnostics.

DAF may also serve as a potential target for molecular cancer therapy [106]. The monoclonal antibody SC-1 has been identified to bind one isoform of DAF which is expressed in gastric carcinoma leading to apoptosis of these cells [33,34,121,122,124]. In pilot clinical trials, patients with poorly differentiated stomach adenocarcinoma of diffuse-type were treated primarily with the SC-1-antibody followed by gastrectomy and lymphadenectomy. A significant induction of apoptotic activity was observed in primary tumours as compared to pre-treatment biopsy material in up to 90% of the cases, and a significant regression of tumour mass up to 50% was noted [123,125]. Moreover, Illert et al. demonstrated that application of SC-1-antibody therapy in nude mice with metastasizing gastric cancer could significantly reduce the number of disseminated tumor cells in the bone marrow [42]. It has also been shown that this therapeutical approach has a comparatively low toxicity in humans [123]. Many studies have tried to find new therapeutic strategies against malignant tumors using monoclonal antibodies against surface antigenes such as DAF [25]. Hsu et al. developed monoclonal antibodies specifically labeling cervical cancer cells. Among those, the antibody labeling DAF showed the widest range of reactivity [41]. Other studies could show that the complement-dependent killing activity of Rituximab, a chimeric anti-CD20 monoclonal antibody, in the treatment of non-Hodgkin’s lymphoma cells is enhanced by additional application of monoclonal anti-DAF-antibody [132]. As DAF may also be physiologically expressed in different cell types, use of bispecific antibodies binding DAF as well as another tumour specific antigen like the renal tumour-associated antigen G250 might be a solution to decrease unwanted side effects [11,33].

Pritchard-Jones et al. could show that additional application of DAF as a cancer vaccine for young osteosarcoma patients treated by myelosuppressive chemotherapy induced significant T-cell proliferation in 71% and antigen-specific gammaIFN secretion in 59% of the cases, and vaccination was well tolerated [94].

It has been shown that DAF is a major cell attachment receptor for Coxsackieviruses. Furthermore, in
vitro studies established that human melanoma cells endogenously express elevated levels of ICAM-1/DAF and were highly susceptible to rapid viral oncolysis by Coxsackievirus A21 infection, whereas ICAM-1/DAF-expressing peripheral blood lymphocytes were refractile to infection. In vivo studies revealed that the tumor burden of immunodeficient mice bearing multiple s.c. melanoma xenografts was rapidly reduced by oncolysis mediated by a single administration of Coxsackievirus A21 [1,6,100,102]. Interactions of the viruses with DAF on the surface of tumour cells seems to exert different functions beyond its immunodeficient activity, binding of viruses, oncogenic tyrosine kinase activation, and specific binding to seven-span transmembrane receptors (CD97) binding for induc- 

9. Conclusion

In conclusion, DAF which is physiologically acting as an inhibitor of the complement system is also broadly expressed in malignant tumours where DAF seems to exert different functions beyond its immunological role such as inhibition of anti-tumour NK cell activity, binding of viruses, oncogenic tyrosine kinase pathway activation, and specific binding to seven-span transmembrane receptors (CD97) binding for induction of cell migration. Therefore, DAF appears to be an interesting target for further study in human malignant tumors and might also become an important target for therapeutic strategies in oncology.

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