Available online at www.sciencedirect.com
ScienceDirect
Molecular Immunology 45 (2008) 169–179
Molecular Immunology
www.elsevier.com/locate/molimm

Non-small cell lung cancer cells produce a functional set of complement factor I and its soluble cofactors

Marcin Okroj [a], Yi-Fan Hsu [b], Daniel Ajona [b], Ruben Pio [b,c], Anna M. Blom [a,*]

[a] *Lund University, Department of Laboratory Medicine, Section of Clinical Chemistry, University Hospital, S-205 02 Malmö, Sweden*
[b] *Division of Oncology, Center for Applied Medical Research, Pamplona, Spain*
[c] *Department of Biochemistry, University of Navarra, Pamplona, Spain*

Received 15 January 2007; received in revised form 12 March 2007; accepted 21 April 2007
Available online 4 June 2007

Abstract

The complement system is important for protection from invading pathogens, removal of waste products and guidance of the immune response. Furthermore, complement can be also targeted to cancer cells. However, membrane-bound inhibitors over-expressed by certain types of tumor cells restrict the cytotoxic activity of complement. Herein we report that non-small cell lung cancer (NSCLC) cells produce soluble complement inhibitors factor I (FI) and C4b-binding protein (C4BP). FI is a serine protease capable of degrading the activated complement components C3b and C4b, whilst C4BP acts as its cofactor. Furthermore, NSCLC cells express membrane-bound regulators and shed membrane cofactor protein (MCP), which shares cofactor function with C4BP. Secretion of FI from NSCLC cells was higher than previously reported for any non-hepatic source and FI produced by these cells could efficiently support cleavage of C3b and C4b. *In vitro* functional assays revealed that additional FI significantly decreased C3 deposition and complement-dependent lysis, particularly when cofactors were added. Our results demonstrate that soluble inhibitors produced by NSCLC cells may provide further protection from complement beyond the level ensured by membrane-bound inhibitors and, as such, contribute to the aggressive phenotype of these lung cancer cells.

Keywords: NSCLC; Tumor; Complement; Cytotoxicity; Immune system

1. Introduction

The complement system plays a role in antimicrobial defense, participates in the removal of immune complexes as well as apoptotic/necrotic cells, and guides adaptive immunity (Walport, 2001). Invading pathogens activate complement either spontaneously through their different surface compositions compared to host (alternative and lectin pathways), or after antibody binding (classical pathway). Key events in complement activation are the formation of the C3 and C5 convertase enzyme complexes, which activate C3 and C5, with subsequent release of the chemoattractants C5a and C3a, and the opsonin C3b. Deposition of C3b on the cell surface leads to complement-dependent cytotoxicity (CDC) through the assembly of the membrane-attack complex (MAC). Complement-dependent cell cytotoxicity (CDCC) is in turn mediated by recognition of surface-deposited C3b by phagocytes and cytotoxic cells. However, misguided or excessive complement activation leads to many autoimmune and inflammatory diseases (Allegretti et al., 2005). Under physiological conditions, complement inhibitors ensure activation of complement only at its desired targets. These inhibitors are either soluble, such as Factor H (FH) and FH-like proteins (FHL's), Factor I (FI) and C4b-binding Protein (C4BP), or membrane bound, such as Membrane Cofactor Protein (MCP, CD46), Decay Accelerating Factor (DAF, CD55), Complement Receptor 1 (CR1, CD35) and Protectin (CD59). Apart from CD59, which inhibits MAC formation, these inhibit C3/C5 convertases by increasing the dissociation of these enzyme complexes and/or by promoting enzymatic cleavage of the activated

Abbreviations: FI, complement factor I; FH, complement factor H; FHL-1, FH-like protein-1; C4BP, C4b-binding protein; NSCLC, non-small cell lung cancer; MCP, membrane cofactor protein (CD46); MAC, membrane-attack complex; CCP, complement control protein; CR1 (CD35), complement receptor 1; ADCC, antibody-dependent cell cytotoxicity; CDCC, complement-dependent cell cytotoxicity; CDC, complement-dependent cytotoxicity; PKC, protein kinase C; DAF, decay accelerating factor (CD55); PS, protein S

* Corresponding author at: Department of Laboratory Medicine, UMAS, Entrance 46, S-205 02 Malmö, Sweden.
E-mail address: anna.blom@med.lu.se (A.M. Blom).

0161-5890/$ – see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.molimm.2007.04.025

complement factors C3b or C4b. Such a cleavage requires FI, which is a plasma serine protease highly specific for arginyl bonds present in C3b and C4b (Tsiftsoglou and Sim, 2004). However, FI is only able to process its natural substrates when appropriate cofactors are present (Blom et al., 2003; Minta et al., 1998; Sim and Laich, 2000). These include C4BP, MCP and CR1, which can support both C3b and C4b cleavage. Additionally, FH promotes exclusively C3b cleavage.

Interestingly, tumor cells can activate complement and deposition of complement components has been shown in tumor tissues of various origins (Jurianz et al., 1999). There are reports describing alternative (Budzko and McConnell, 1976), lectin (Fujita et al., 1995) and classical (Jurianz et al., 1999) complement pathway activation by tumor cells. However, anti-tumor antibodies in cancer patients are often detected at low titer or have low affinity (Jurianz et al., 1999). The introduction of mAb-based anticancer drugs such as Rituximab has prompted renewed interest in complement activation by tumor cells (Stern, 2005). Analysis of susceptibility to antibody-mediated killing of primary non-Hodgkin's lymphoma revealed that *in vitro* cell sensitivity to CDC was the best predictor of clinical response to antibodies (Manches et al., 2003). This indicates the importance of complement in the elimination of tumor cells. Although mAb therapy offers good potential for treating cancer, one serious limitation is the over-expression and shedding of membrane-bound complement inhibitors by many tumor cells (Gorter and Meri, 1999). Furthermore, cancer cells neutralize complement damage by removing MAC from their surface by internalization and shedding (Donin et al., 2003). Additionally, we have reported that many non-small cell lung cancer (NSCLC) cell lines secrete the soluble complement inhibitors FH and FHL-1 (Ajona et al., 2004). In the present study we show that some NSCLC cells are also able to secrete FI, together with cofactors C4BP and shed CD46. Moreover, we analyze the activity and functional importance of this protease and its soluble cofactors for their host cells.

2. Materials and methods

2.1. Cell culture

Lung cancer cell lines were obtained from the American Type Culture Collection (Manassas, VA). Non-malignant, immortalized lung epithelial cells BEAS-2B were a gift of Dr Benjamin Stoeckler (Regensburg University, Germany). Cells were cultured in RPMI 1640 supplemented with L-glutamine, 10% fetal calf serum, streptomycin and penicillin (Invitrogen, Carlsbad, CA) and passaged by trypsinization (adherent cell lines). For collecting the conditioned media, cells were washed with PBS and cultured for 48 h in serum-free Optimen medium (Invitrogen).

2.2. Proteins and human sera

Human plasma C4BP (Dahlbäck, 1983), FH (Blom et al., 2003), FI (Crossley and Porter, 1980) and human recombinant CD46 (Blom et al., 2001b) were purified as described in their respective references. C3b and C4b were purchased from Advanced Research Technologies (San Diego, CA). C4BP, C4b and C3b were labeled with ^{125}I using the chloramine T method. The specific activity was 0.4–0.5 MBq/μg of protein. Normal human serum (NHS) was collected from healthy volunteers and pooled. C6-deficient serum was provided by Dr. Anders Sjöholm from Lund Hospital, Sweden, with the permission of the local ethical committee.

2.3. Antibodies

The monoclonal antibody MK104 directed against complement control protein domain 1 (CCP1) of C4BP α-chain blocks the binding of C4b, inhibiting the function of C4BP. Monoclonal antibody MK67 is directed against CCP4 of the α-chain of C4BP (Blom et al., 2001a). Polyclonal antibody PK9008 was raised in rabbits against C4BP–PS complex isolated from human plasma. OX21 is a monoclonal antibody against FI and was generously provided by Prof. Sim, Oxford University. Anti-human Factor I Quidel #1–a function-blocking antibody against FI, was purcharsed from Quidel (San Diego, CA). PK9205 is a rabbit polyclonal antibody against FI, raised in-house against the protein isolated from human plasma. MK21 is a mouse monoclonal antibody directed against the γ-carboxyglutamic acid domain of PS (gift of Prof. Dahlbäck, Lund University). Rabbit polyclonal antibody against FH was from Quidel whilst OX23 and OX24 are monoclonal antibodies against FH raised at Oxford University. Monoclonal function-blocking antibodies against CD46: MEM-258 and GB24 were purchased from Immunotools (Frieosoythe, Germany) or generously provided by Prof. Atkinson (Washington University), respectively. Rabbit polyclonal antibody raised against CD46 (Elward et al., 2005) was kindly provided by Dr. Spiller, Cardiff University. Function-blocking antibody BRIC229 against CD59 was obtained from IBGRL (Bristol, UK). Secondary antibodies against mouse or rabbit immunoglobulins, unlabeled or conjugated with HRP or FITC, were from DakoCytomation (Glostrup, Danmark).

2.4. RT-PCR

RNA was purified from lung cancer cells using the Ultraspec Total RNA Isolation Reagent (Biotecx, Houston, TX). Analysis of mRNA expression was performed by RT-PCR using the following primers: C4BP α-chain, sense CTTGATCGCTGCTCTGTTGCCTG, antisense CGCTGTGCCTTCCATTCCTGATG; C4BP β-chain, sense TCCTGATCCTGTGCTGGTGAATG, antisense AAGTTCTCCATGGCTTCGCAGAG; FI, sense TAACGGAACATGCACAGCCGAAG, antisense CACAGATGCAAAGCCTGCACAGC; PS, sense CACTGGTGTTATGCTTGCCTTGG, antisense AATGGAACATCTGGAAGGCCACC.

2.5. Immunoprecipitation of C4BP

Medium conditioned by lung cancer cells was concentrated 10-fold and 100 ng of the polyclonal rabbit antibody PK9008 was added. Samples were rotated end-over-end overnight at 4 °C. Afterwards, 30 μl of a protein A-sepharose (Sigma, St.