Mini-Review

Chitinase-3-like-1/YKL-40 as marker of circulating tumor cells

Gerhard Hamilton[1], Barbara Rath[1], Otto Burghuber[2]

[1]Ludwig Boltzmann Cluster of Translational Oncology, A-1090 Vienna, Austria; [2]Ludwig Boltzmann Institute for COPD and Respiratory Epidemiology, 1140 Vienna, Austria

Correspondence to: Dr. Gerhard Hamilton. Ludwig Boltzmann Cluster of Translational Oncology, c/o Balderichgasse 26/13, A-1170 Vienna, Austria. Email: gerhard.hamilton@toc.lbg.ac.at.

Abstract: *Ex vivo* expansion of circulating tumor cells (CTCs) of small cell lung cancer (SCLC) patients enabled systematic screening of secreted cytokines. Permanent CTC cultures of different patients shared secretion of chitinase-3-like-1 (CHI3L1)/YKL-40, known to be upregulated in a range of tumor entities and to be associated with increased metastasis and decreased survival. This protein lacks enzymatic activity and its mechanism of promoting tumor dissemination has not been resolved. Results from SCLC CTC cultures suggest CHI3L1 as marker and important effector of tumor cell dissemination in the peripheral blood. Furthermore, this protein may link chronic inflammation of the lung, chronic obstructive pulmonary disease (COPD) and lung cancer.

Keywords: Small cell lung cancer (SCLC); circulating tumor cells (CTCs); secretome; chitinase-3-like-1 (CHI3L1); YKL-40; chronic obstructive pulmonary disease (COPD); prognostic marker

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Introduction

Lung cancer is the leading cause of tumor-associated death worldwide (1). Small cell lung cancer (SCLC) is a neuroendocrine and highly malignant subtype of lung cancer, accounting for approximately 15% of cases, which exhibits a low survival rate at disseminated stage. In contrast to non-small lung cancer (NSCLC), targeted therapy directed to specific oncogenes is not available for SCLC and platinum-based combination chemotherapy with etoposide and second-line topotecan are standard care (2). SCLCs invariably show inactivation of p53 and retinoblastoma (Rb) and in the absence of these two tumor suppressors several alternative growth factor pathways induce vigorous progression (3). Despite an initial high response rate, tumors relapse early and are not amenable to effective further therapy. Progress of research on SCLC is hampered by availability of tumor material, as in extended disease no material is collected after initial confirmation of the diagnosis by fine-needle biopsies. Alternatively, so-called liquid biopsies consist of the detection of circulating genetic material or enrichment of the diversified circulating tumor cells (CTCs) using blood samples (4). In most cases CTCs are scarce and investigations are restricted to single-cell genetic analysis, detection of surface markers or characterization of a limited number of cytokines by the EPISPOT assay which detects proteins secreted/released/shed from single epithelial cancer cells (5).

Ex vivo culture of SCLC CTCs

Cancer patients featuring high CTC counts (> several hundred CTCs/7.5 mL blood) may allow for the initiation of CTC cultures *in vitro* or as xenotransplants (CDX) in immunocompromised mice. This has been demonstrated in a few instances in breast and colon cancer and for CDXs in a number of SCLCs. In detail, several heterogenous permanent CTC cultures were published for breast cancer, one permanent CTC line of a large number of colon cancer patients and several SCLC CDX, the latter representing the clinical course and chemosensitivity of the parent tumors (6-8). In contrast to other tumor entities, including NSCLC, SCLC patients may exhibit excessive numbers of CTCs at advanced stage and may allow for *ex vivo* expansion

of these cells under regular tissue culture conditions. In fact, we have obtained three permanent CTC cell lines from advanced and relapsed SCLC (9). Cell culture supernatants were screened for expression of cytokines and chitinase-3-like-1 (CHI3L1)/YKL-40 was detected as commonly expressed factor, among others (manuscript submitted). The alternative designation of CHI3L1, namely YKL-40, is based on the first three N-terminal amino acids, tyrosine (Y), lysine (K) and leucine (L) and the apparent molecular weight of YKL-40.

CHI3L1/YKL-40 as tumor marker

Human chitinase-like glycoproteins are known to be expressed in several types of solid tumors that include breast, colon, kidney, SCLC, ovarian, prostate, endometrial cancer, malignant melanoma, glioblastoma, and Hodgkin's lymphoma (10). Levels of circulating CHI3L1 are increased in many malignancies, including cancers involving the lung, prostate, colon, rectum, ovary, kidney, breast, glioblastomas, and malignant melanoma. Numerous studies have correlated elevated serum levels of CHI3L1 with poor prognosis and low survival in patients suffering from these malignancies (10). Furthermore, in breast and colon cancer it was shown that increased CHI3L1 levels correlate with tumor grade and poor differentiation of cancer cells (11,12). CHI3L1 plays an important role in prostate cancer progression (13).

CHI3L1 was significantly upregulated in NSCLC tissues and CHI3L1 protein overexpression and high microvascular density (MVD) were significantly associated with tumor relapse and an unfavorable overall survival (14). Increased expression of CHI3L1 and increased MVD was associated with metastasis and a worse prognosis in clear cell renal cell cancer (15). Multivariate analysis identified CHI3L1 as an independent prognostic predictor for overall survival and disease-free survival of hepatocellular carcinoma patients (16). CHI3L1 expressed in colon cancer cells was found to promote cancer cell proliferation, macrophage recruitment and angiogenesis (17). Furthermore, CHI3L1 plays an important role in the regulation of malignant transformation and local invasiveness in gliomas (18). Elevated serum levels of CHI3L1 in patients with cervical cancer positively correlated with vascular endothelial growth factor (VEGF) expression, MVD and cancer metastasis and this protein was induced during pulmonary melanoma metastasis (19,20). In summary, the CHI3L1 protein is expressed in many types of cancer cells and its highest plasma levels have been found in patients with metastatic disease, short recurrence/progression-free intervals, and low overall survival (21-23).

In particular, serum CHI3L1 was evaluated in 131 patients with SCLC (24). Twenty-two percent of the patients with limited disease and 40% of the patients with extensive disease had elevated serum concentrations of CHI3L1. The median survival was 5.1 months for patients with elevated serum CHI3L1 and 9.0 months for patients with normal serum CHI3L1. Patients with elevated serum CHI3L1 had increased hazard for death within the first 6 months of chemotherapy.

Functional significance of CHI3L1 expression

CHI3L1 is a secreted 40 kDa glycoprotein which lacks chitinase activity due to mutations within its active site (25). Although the polysaccharide chitin is not found in mammals, several proteins homologous to bacterial, fungal, and plant chitinases are expressed, obviously fulfilling different functions (10). CHI3L1 interacts with glycosaminoglycans, such as heparin and hyaluronan, and binds to collagen type I, II, and III. When CHI3L1 expression was inhibited in a glioblastoma cell line, a reduction in VEGF was observed and vice versa, blockade of VEGF induced the expression of CHI3L1 (26). CHI3L1 suppressed E-cadherin but increased matrix metalloproteinase-9 (MMP9) and cell motility, all of them essential features of tumor cell invasion (27). Furthermore, CHI3L1 was found as migration factor for primary astrocytes (28). CHI3L1 has been linked to activation of the AKT pro-survival (anti-apoptotic) signaling pathway and enhances tumor survival in response to irradiation which is frequently applied in SCLC (26).

CHI3L1 plays an important role in the regulation of malignant transformation and local invasiveness in gliomas (18). CHI3L1 was highly expressed compared to normal brain tissue and its elevation in the serum predicted a poor clinical outcome (29,30). Targeting CHI3L1 with neutralizing antibodies has been proven effective as a treatment in animal models of glioblastoma (31). CHI3L1 suppression by shRNA reduced glioma cell invasion, anchorage-independent growth and increased cell death in response to several anticancer drugs, including cisplatin, etoposide and doxorubicin (18). Overexpressed proteins in the glioma secretome included metalloproteinases, cathepsins, osteopontin, and CHI3L1, among others (32). The CHI3L1 protein was significantly elevated in human serum samples from all types of lung cancers and recombinant CHI3L1

stimulated proliferation and growth of Lewis lung carcinoma cells (33). In conclusion, expression of CHI3L1 is linked to stimulation of proliferation, motility, invasive capacity and drug resistance of tumor cells by mechanisms poorly characterized so far.

Discussion and conclusions

CHI3L1 is detectable in a variety of normal cells, including macrophages, neutrophils, epithelial cells, smooth muscle cells, and chondrocytes, and its expression is stimulated by a number of cytokines, including IL13, IL6, IL1β, and IFNγ (20,34) Elevated serum levels of CHI3L1 have been associated with a negative outcome in a number of nonmalignant diseases such as inflammation and asthma (34,35). Thus, in clinical determinations of serum CHI3L1 in patients, secretion from normal tissues would have been to be considered. Additionally, CHI3L1 may link inflammation of bronchial tissue, chronic obstructive pulmonary disease (COPD) and tumor and provide an explanation for COPD as risk factor of lung cancer (12,36). The presence of CTCs as examined by an ISET filtration-enrichment technique (isolation by size of tumor cells) in COPD patients (3% positivity) was reported to precede the clinical detection of early stage lung cancer by 1-4 years (12).

Clearly, the primary tumor tissue constitutes a source of CHI3L1 which is likely to be spilled out to the peripheral circulation and represents a marker of tumor burden. The highly ubiquitous CHI3L1 protein may be involved in local invasion of primary tumors. However, CHI3L1-positive tumor cells, exhibiting altered regulation of VEGF and MMP9, decreased expression of cadherin and increased cell motility, display the combination of characteristics required to generate metastases as CTCs. Preclinical research and experimental models using glioma cells support the role of CHI3L1 in tumor dissemination and drug resistance (26). Our results demonstrating CHI3L1 as part of the secretome of *ex vivo* expanded SCLC CTCs point to these disseminated tumor cells as source of this protein directly produced in blood. Quantitatively, serum concentrations of CHI3L1 have been reported to be increased by approximately 50 ng/mL in stage I/II and 200 ng/mL in stage II/IV colon cancer, respectively, compared to healthy controls (17). Whereas in tumors with CTCs counts of several cells/mL blood these concentrations of CHI3L1 are not expected to be produced by CTCs, in tumors such as SCLC which can exhibit CTC counts of more than several hundred tumor cells/mL blood, most of this cytokine may actually be contributed by CTCs (8). Ubiquitous expression of CHI3L1 in a range of solid tumors, as indicator of early dissemination and lower overall survival, may at least partially be due to CHI3L1-positive CTCs. Therefore, it should be investigated whether CHI3L1 constitutes a general CTC-associated marker aside from SCLC and whether CTC counts parallel the serum concentrations of this antigen (37). Experimental animal data reveal a therapeutic modality demonstrating decreased tumor dissemination in response to administration of chitosan nanoparticles which may capitalize on the conserved binding to CHI3L1 (38). In conclusion, CHI3L1 seems to constitute an important marker for CTCs which has functional significance and may represent a putative target for interfering with tumor cell dissemination. The current ambiguous mechanism of CH3L1 in metastatic spread may reside in its expression and utilization by CTCs.

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