Review

Markers of small cell lung cancer

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Abstract

Lung cancer is the number one cause of cancer death; however, no specific serum biomarker is available till date for detection of early lung cancer. Despite good initial response to chemotherapy, small-cell lung cancer (SCLC) has a poor prognosis. Therefore, it is important to identify molecular markers that might influence survival and may serve as potential therapeutic targets. The review aims to summarize the current knowledge of serum biomarkers in SCLC to improve diagnostic efficiency in the detection of tumor progression in lung cancer. The current knowledge on the known serum cytokines and tumor biomarkers of SCLC is emphasized. Recent findings in the search for novel diagnostic and therapeutic molecular markers using the emerging genomic technology for detecting lung cancer are also described. It is believed that implementing these new research techniques will facilitate and improve early detection, prognostication and better treatment of SCLC.

Introduction

Lung cancer is the most common cancer world over. It is classified into small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). They occur with a frequency of 20% and 80% respectively [1]. The aggressive nature of SCLC with frequent and early metastases accounts for a dismal 5-year survival rate of <5% with current standard therapies. Metastases initially occur in the lymph nodes and thereafter in other organs such as the lung itself, liver, adrenal glands, brain, bone, and bone marrow. The antigenic profile of SCLC coincides mainly with the neuroendocrine cells because of similar origin. Early detection of SCLC is challenging mainly due to the lack of adequate serum tumor markers. In spite of aggressive therapy available today, the prognosis of lung cancer patients is generally very poor. Therefore, the development of novel diagnostic techniques to identify lung can-

cer is important to facilitate earlier diagnosis of primary or recurring cancers leading to more effective treatment and improved prognosis [2]. Various molecules detectable in the serum, useful as putative markers of the disease include chromogranin A (CgA), pro-gastrin releasing peptide (ProGRP) and neuron-specific enolase (NSE; an γ-γ isoform of the ubiquitous enolase enzyme), cytokeratin 19 marker CYFRA 21-1 etc. The tumor markers demonstrate great significance in the neuroendocrine differentiation of lung cancer. Chromogranin A (CgA), a 49 kDa acidic-soluble protein ubiquitously present in neuroendocrine tissues, serves as a suitable circulating marker of neoplasms of neuroendocrine origin. Release of this secretory protein in the serum of patients suffering from SCLC has been reported. The ability of serum CgA to distinguish neuroendocrine and non-neuroendocrine tumors either *in situ* or by serum level titration has also been suggested

[3]. In a report aimed to determine the diagnostic performance of an immunoradiometric assay of CgA in SCLC and to use it as a method for discrimination from neuron-specific enolase (NSE), it has been found that CgA assay shows better diagnostic sensitivity than NSE in SCLC (61% versus 57%), especially in limited disease. In contrast, NSE reflected disease extent more accurately than CgA. It has also been shown that the CgA assay is not affected by hemolysis whereas NSE serum levels greatly increased in hemolysed sera. CgA assaying by this method is a reliable procedure in the diagnosis of SCLC whereas, NSE is suitable marker of choice in staging and monitoring of the disease [3,4].

Recent reports show the expression of selected neuroendocrine markers (CgA, NSE and synaptophysin) confirming the neuroendocrine origin of SCLC and also found the content of two anti-neoplastic cytokines, IL-2 and IL-12 in the tumors [5]. Data on the lowered secretion of the two cytokines, IL-2 and of IL-12 at the time of diagnosis may represent a prognostic factor for survival in SCLC [5].

Gastrin-releasing peptide (GRP), a gut hormone, is present in the nerve fibres, brain and neuroendocrine cells in the fetal lung [6,7]. It was originally isolated from the porcine stomach and is the mammalian counterpart of bombesin. In spite of the elevated levels of plasma GRP in the patients suffering from SCLC, its regular use as a diagnostic marker is not preferred because of its unstable nature in the serum [3].

Various studies have shown that ProGRP is superior to other markers in its ability to differentiate SCLC and NSCLC. ProGRP fragment (31–98) is referred to as a common region to three types of cloned human ProGRP molecules [7-9]. Serum proGRP (31–98) levels, measured by ELISA, and the expression of proGRP as well as GRPR mRNA in SCLC tumor tissues, investigated by reverse transcription-nested polymerase chain reaction (RT-PCR) amplification, in the individuals with SCLC have been reported [10]. Expression of RT-PCR based amplification of transcripts in cancer but not in normal non-neoplastic cells suggests its use for the sensitive detection of rare disseminated or exfoliated cancer cells to improve cancer staging and early detection. RT-PCR assays for seven neuroendocrine marker transcripts including neural cell adhesion molecule (NCAM), protein gene product (PGP 9.5), gastrin, gastrin receptor, synaptophysin, preprogastrin-releasing peptide (preproGRP) and GRP-receptor for the detection of rare exfoliated tumor cells in peripheral venous blood and sputum samples from patients with lung cancer are reported. Amplification of preproGRP transcripts from clinical samples indicates this as a sensitive and specific assay to detect disseminated or exfoliated

lung cancer cells either in peripheral blood or sputum samples [11].

Differential immunization for antigen and antibody discovery technology (DIAAD)

Another very recent approach towards the development of SCLC specific marker is through the antigenic subtraction of neuroblastoma cells. Multiple attempts to generate monoclonal antibodies (MoAbs) against SCLC-specific antigens have failed due to their cross-reactivity with neuroendocrine tissues or normal immune cells.

The concept of DIAAD helps to generate immune response that can be effectively focused on cancer cells. DIAAD follows protocol of induction of tolerance and immunity, conducted in sequential manner to "biologically subtract" the immune response of dominant antigens expressed on the normal cells. This biological subtraction is gained by first eliminating the immune response to the normal healthy cells followed by immunization with the diseased cells [12]. This protocol followed in the laboratory animals directs the immune response specifically towards the antigens expressed in the target and not in the normal cells. Enormous efforts have been made to generate SCLC-specific MoAbs. The DIAAD method allows getting rid of B-cells carrying receptors directed against undesired molecules present on the normal cells prior to the generation of B-cells specific to the target cells [12]. Recently, *Kruger et al* have been able to generate four MoAbs that can recognize tumor-associated antigens expressed on SCLC cells. The antibodies do not cross-react with most of the other undesired cancer cells as well as normal cells. Moreover, the SCLC antigen is present in the sera of patients in a concentration that exceeds 6 logs in comparison with the normal sera, suggesting its role as a secretory marker for the early detection of the disease. The DIAAD technology is able to generate antibodies that detect antigens with broad chemical diversity and supports the utility of these antibodies for the treatment of patients with SCLC as well as in the diagnosis of the disease.

DNA markers
Another major focus in the generation of a sensitive and tumor specific marker in the lung cancer patients is the plasma DNA. The accumulation of genetic alterations is seen very commonly in lung cancers. Amongst microsatellite (MS) alterations, primary lung cancer seldom shows microsatellite instability but more often is depicted by frequent loss of heterozygosity. The levels of free-circulating DNA in the plasma are known to be higher in patients than in healthy controls. Plasma DNA analysis is suggested to be of significance for the prognosis of the disease. Attempts have been made to study and evaluate the sensitivity and specificity of the large group of microsatel-

lites for the detection of molecular genetic alterations in the plasma and paired tumor DNA of lung cancer patients. The method is useful for monitoring lung cancer progression at different stages of the disease [13].

Reports by *Chen et al* and *Gonzalez et al* show the best sensitivity for characterizing plasma DNA in SCLC at 71% using three molecular markers (ACTBP2, UT762 and AR). The sensitivity in other studies ranged from 23–50% in both SCLC and NSCLC using the same markers as alone or 4–5 markers targeting mainly chromosome arm 3p [14,15].

Small-cell lung cancer (SCLC) cells express various markers of neuronal differentiation associated with deficient activity of the neuron-restricted silencer factor (NRSF). The glycine receptor (GlyR) alpha1 subunit gene, GLRA1, contains a sequence motif for NRSF binding (NRSE) within its 5' UTR, which serves as a cellular surrogate marker for NRSF activity. Expression of GLRA1 in non transformed cells is largely restricted to cells in the spinal cord, retina and brain stem. NRSF-mediated suppression of an expression construct harboring the NRSE of the GLRA1 (GLRA1 NRSE) gene has been shown to be impaired in three of four 'classic' SCLC cell lines, whereas exogenous overexpression of NRSF in NRSF-deficient SCLC cell lines has been reported to reconstitute the silencing of the reporter plasmid [16]. Most interestingly, induction of apoptosis in SCLC cells is reported upon reconstitution of NRSF expression, indicating inhibition of NRSF activity as a crucial step in the carcinogenesis of a subgroup of SCLC [16].

The human achaete-scute homologue 1 (HASH1, ASCL1), a lineage-specific basic helix-loop-helix member of the achaete-scute family, is essential for the generation of pulmonary neuroendocrine (NE) cells during lung development. In small cell lung cancer (SCLC), the gene is highly expressed and the expression of HASH1 correlates with NE features found in SCLCs. A highly sensitive reverse transcription-PCR method for quantifying HASH1 mRNA in clinical samples, using real-time fluorescence resonance energy transfer technology has shown marked and measurable differences existing between SCLCs and other lung tissues (either NSCLC or normal bronchus). This method contributes to diagnosis based on molecular profiling of tumors using HASH1, ASCL1 as a marker for SCLC [17].

Role of kinases
Receptor tyrosine kinases (RTKs) initiate specific intercellular signaling pathways in response to binding of extracellular growth factors and are known to play an important role in regulating normal and cancer cell growth and differentiation. Overexpression of receptor type tyrosine kinase is associated with an aggressive tumor phenotype and poor outcome of various cancers. A tyrosine kinase, c-kit (CD117) oncoprotein is expressed in a variety of tumors including mastocytosis, germ cell tumors, gastrointestinal stromal tumors (GIST) and SCLC. Various analyses have consistently shown that all SCLCs express abundant stem cell factor (SCF) mRNA, and since SCF is the c-kit ligand, it suggests the possible involvement of an autocrine mechanism in the development of c-kit positive SCLCs [18] and LCNECs (large cell neuroendocrine carcinoma) [19]. Reports that over 70% of SCLC cell lines and tumor co-express c-kit and its ligand, SCF suggest that they are part of autocrine loop in these cells [19-21]. SCF appears to be an important growth factor for SCLC cells, because growth suppression has been observed when a kinase defective c-kit was introduced into a c-kit/SCF expressing cell line [22]. Cell lines expressing the c-kit gene are also shown to express either the L- and N-myc genes, however, those cell lines that express the c-myc gene do not express the c-kit gene. Heterologous expression of c-myc correlates with a marked down-regulation of c-kit expression in several small lung cancer cell lines when transfected with a c-myc expression vector. Regulation of c-kit expression by the myc gene family may be partly responsible for the differing biological properties of cell lines and tumors which express N- and L-myc versus those that express c-myc [20]. STI571 is a small synthetic molecule that shows selective inhibition of tyrosine kinase activity and function through competitive inhibition of ATP binding of the enzymes since most of the SCLC expresses c-kit tyrosine kinase oncoprotein, STI571 might be a potential therapeutic agent in the treatment of SCLC [23]. RTKs are a target for novel therapies currently being investigated.

Epidermal growth factor receptor (EGFR) inhibitors and c-Kit inhibitors and c-Met inhibitors are being used. Even though the RTK inhibitors provide a novel mechanism, it is important to realize that lung cancer etiology is a complex process, and eventually standard chemotherapy may need to be used in conjunction with these novel therapies to make an important difference in response rates. The levels of carcinoembryonic antigen (CEA) and the activities of creatine kinase isoenzyme BB (CK-BB) in lung cancers have been reported in the literature. Increased levels of CEA are observed mostly in patients with NSCLC, while enhanced activities of CK-BB in patients with SCLC. A relationship between enhanced levels of CEA or CK-BB and advanced carcinoma has also been demonstrated. The increased values of studied markers seem to indicate the limited possibility of surgical treatment and they are also important in prognosis after the resection of lung tissue [24].

Telomerase activity

Telomerase is an enzyme that adds hexameric TTAGGG nucleotide repeats onto the ends of vertebrate chromosomal DNAs (i.e., telomeres) to compensate for losses that occur with each round of DNA replication. It has been suggested that immortalized cells (including some, but probably not all, cancer cells) continue to proliferate indefinitely because they express telomerase. Cancers that exhibit high levels of telomerase activity, such as all of the small-cell lung cancers are likely to consist mainly of immortal cells. Telomerase activity may be useful both as a diagnostic marker to detect the existence of immortal lung cancer cells in clinical materials and as a target for therapeutic intervention [25].

Significance of apoptotic cell death pathway

Clonal expression and tumor growth results not only from the acceleration of intrinsic proliferation but also due to escape from apoptotic cell death. Alterations in the normal physiological apoptotic pathway results in a significant survival advantage of the tumor cells even in the unfavorable conditions. The role of Fas cell death receptor pathway is well established in the inhibition of apoptosis. FasR (a member of tumor necrosis factor superfamily) interacts with FasL to trigger cells to undergo apoptosis. Evidences suggest that the Fas expression is clearly decreased in lung tumor, regardless of the histological type, with clear over expression of FasL in the high grade SCLC tumors [26].

Hopkins-Donaldson *et al* have demonstrated the resistance of small cell lung cancer cell lines to FasL and TRAIL-induced apoptosis, which could be explained by an absence of Fas and TRAIL-R1 mRNA expression and a deficiency of surface TRAIL-R2 protein [27]. Reduced levels of Fas, TRAIL-R1 and caspase-8 mRNA molecules in SCLC tumors compared to NSCLC tumors justify the resistance of SCLC cells to apoptosis mediated by death receptors. RT-PCR studies on loss of expression of caspase-8 in most of SCLC cell lines but with retained expression in all 22 non-SCLC (NSCLC) lines tested have been reported. Recent reports indicate that caspase-8 expression is lost via a combination of promoter methylation and allelic loss in a subset of neuroblastomas [28].

SCLC is surrounded by an extensive stroma of extracellular matrix (ECM) at both primary and metastatic sites. Adhesion of SCLC cells to matrix enhances tumorigenicity and confers resistance to chemotherapeutic agents as a result of beta1 integrin-stimulated tyrosine kinase activation, suppressing chemotherapy-induced apoptosis. SCLC may create a specialized microenvironment, and the survival of cells bound to ECM could explain the partial responses and local recurrence of SCLC often seen clinically after chemotherapy. Strategies based on blocking beta1 integrin-mediated survival signals may represent a new therapeutic approach to improve the response to chemotherapy in SCLC [29].

Conclusions

In conclusion, availability of tumor markers such as serum CgA, GRP and proGRP as determinants of disease in SCLC may improve the overall outlook. DIAAD's capacity to elicit a robust response to broad range of non-dominant cancer antigens uniquely expressed on SCLC allows generation of specific MoAbs against SCLC antigens. These specific MoAbs may serve as a crucial biomarker for early diagnosis of the disease, as well as for the therapeutic intervention for SCLC. Presently, the diagnosis of SCLC is made at an advanced stage with a dismal outlook, however, its early detection may promise a cure. Thus, the addition of an early new diagnostic test for SCLC will indisputably save many lives and also help in the prolonged survival of patients suffering from this devastating disease.

Competing interests

None declared.

Authors' contribution

SKS conceived the idea besides holding the major contribution in editing and giving the final shape to this article. TKT contributed in literature search and the preparation of the draft of the article.

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