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Review

Novel approaches to the treatment of small-cell lung cancer

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Abstract. Small-cell lung cancer (SCLC) is characterized by its initial responsiveness to chemotherapy and the appearance of early metastases. Although combination chemotherapy, in some instances together with radiation, has improved the prognosis of this disease, in most patients SCLC ultimately recurs in a drug-resistant form. Several new strategies for the eradication of SCLC are being explored at the preclinical level. The identification of selective target molecules on the surface of SCLC cells, together with the progress made in antibody engineering, have provided new generations of antibodies and immunoconjugates as well as growth factor antagonists and inhibitors. In addition, recent advances in understanding the biology of SCLC have stimulated new investigations searching to counter the molecular basis underlying the increased proliferation and the apoptosis deficiency of SCLC cells. This can be achieved using antisense oligodeoxynucleotides that repress the expression of growth factor receptors and anti-apoptosis genes, or by gene replacement to compensate for the loss or inactivation of tumor suppressor genes.

Key words. Antisense; antibodies; growth factor inhibitors; chemotherapy; gene therapy; radiation therapy; small-cell lung cancer.

Lung cancer is the leading cause of cancer-related deaths, and its incidence continues to rise worldwide [1]. This cancer can be classified in two major histological types. Roughly 80% are non-small-cell lung cancers (NSCLCs), comprising adenocarcinoma, large-cell carcinoma, and squamous cell carcinoma. Most of the remaining cases are classified as small-cell lung cancer (SCLC), which has a different biological behavior, history, and treatment. SCLC is a very aggressive disease, characterized by rapid growth and early dissemination. In the seventies, SCLC was shown to be responsive to chemotherapy. While the median survival before the introduction of chemotherapy was 2–4 months [2], combination chemotherapy has increased survival about fourfold and resulted in long-term survival or cure in a small proportion of patients [3]. In most patients, however, the tumor relapses in a form resistant to any further cytotoxic treatment.

During the past decade, numerous studies have addressed the molecular and genetic mechanisms underlying the development and progression of malignant tumors. In the case of SCLC, many abnormalities have been identified, which allow the development of approaches that specifically target the molecular basis of this disease. Together with the improvements made in the field of drug development and delivery, this has opened new avenues for more effective treatment of SCLC, and may help to improve the treatment outcome of this hitherto incurable disease. This review will summarize the most prominent advances in the treatment of SCLC provided by preclinical and clinical studies during the last decade, which deserve further investigation:

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(i) recent improvements in chemo- and radiation therapy; (ii) approaches to target cell surface antigens, autocrine growth factors and their receptors, and modulators of drug resistance; (iii) approaches to target the molecular basis of increased proliferation and apoptosis deficiency of tumor cells, such as the use of bcl-2 antisense oligodeoxynucleotides or the replacement of inactivated or lost tumor suppressor genes. A summary of these issues is shown in figure 1.

Combination chemotherapy

Back in the sixties it was shown that local therapy, either surgery or radiation alone, had no significant impact on the survival of SCLC patients [4, 5]. In the seventies, several chemotherapeutic drugs with activity against SCLC were identified, including nitrogen mustard, cyclophosphamide, methothrexate, doxorubicin, etoposide, procarbacine, and vincristine. It was also shown that a combination of up to three drugs yields better results than individual drugs used alone or sequentially. In the eighties, one new active drug, cisplatin, was identified, and several analogs of older agents, with similar modes of action but somewhat different toxicity profiles, were reported, including ifosfamide, epirubicin, teneposide, vindesin, and carboplatin. Based on many clinical studies, combination therapy with cisplatin and etoposide, or cyclophosphamide, doxorubicin and vincristine, both combina-

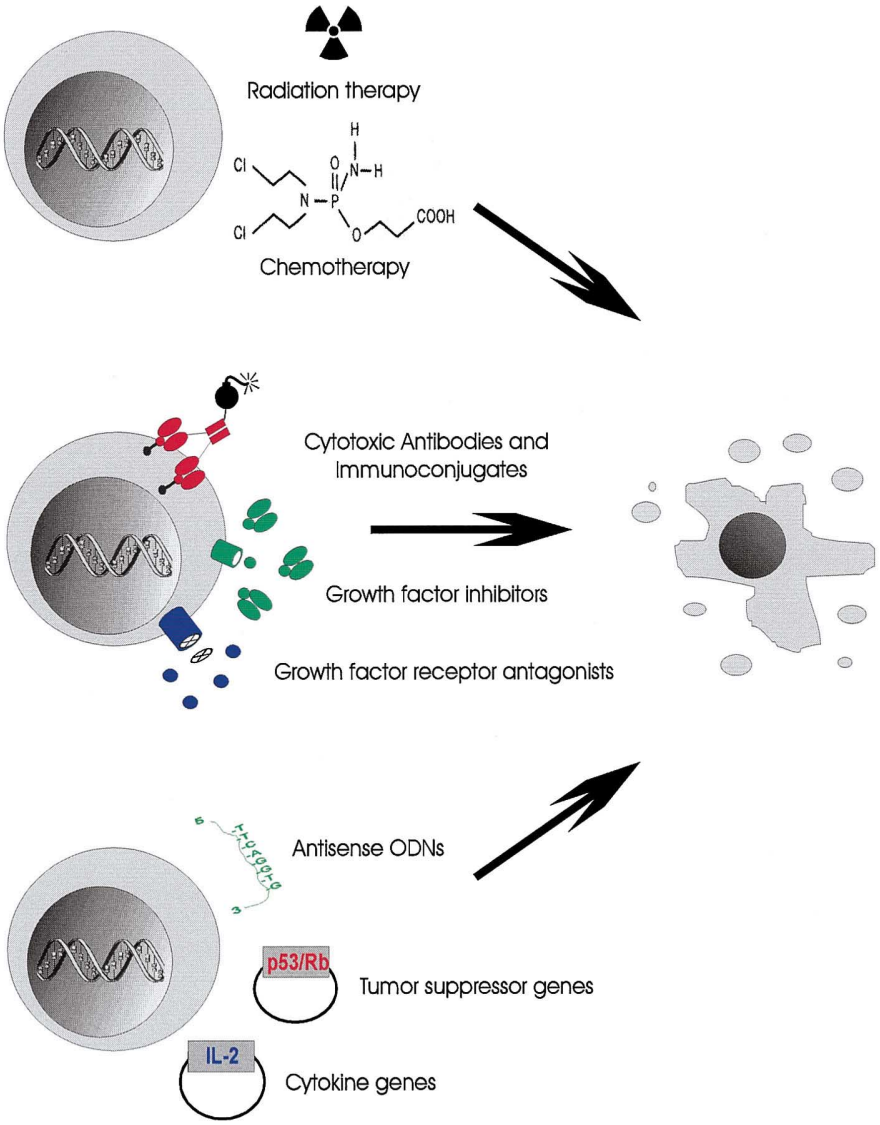

Figure 1. Overview of the various treatment approaches discussed in this review.

tions given for four to six cycles of treatment at 3-weekly intervals, has become the standard therapy of this disease [6].

Advances in chemotherapy reached a plateau in the eighties, despite many clinical trials testing further strategies to improve the results. Among these strategies were the intensification of drug delivery by weekly chemotherapy [7], the use of higher than conventional doses of standard chemotherapy, with or without hematopoietic growth factors [8, 9], the use of the anticoagulant warfarin together with chemotherapy [10], interferon maintenance [11], and the use of megestrol acetate to increase weight gain together with chemotherapy [12].

Currently, several new agents have been identified as having good activity in SCLC, including taxanes [13], gemcitabine [14], vinorelbine [15], and topotecan [16]. However, how these new agents should best be integrated in combination regimens and whether their inclusion will improve the outcome of SCLC have not yet been determined.

Radiation therapy

The role of thoracic radiotherapy for limited-disease SCLC, i.e., SCLC confined to one hemithorax, has long been controversial. A meta-analysis of 13 randomized trials has settled this issue by demonstrating a moderate 5% survival benefit at 3 years for the use of combined chemotherapy and radiation therapy [17]. A recent publication from a large study showed that hyperfractionation compared to conventional fractionation gave a 5-year survival benefit from 16 to 26% [18]. For patients responding to therapy, relapses in the brain may occur frequently. To decrease the rate of brain relapses, many investigators suggested the use of prophylactic cranial irradiation. While it has been undisputed that such prophylactic cranial irradiation does reduce the occurrence of brain metastasis, the potential for neuropsychiatric side effects and lack of proof of a survival benefit left many physicians doubtful about the standard use of this treatment. Recent studies, however, failed to demonstrate an impairment of cognitive function and a meta-analysis demonstrated a significant survival benefit in complete responders from 15 to 20% at 3 years, suggesting prophylactic cranial irradiation should be offered to patients in complete remission [19–21].

Targeting cell surface antigens

Antibodies recognizing surface antigens selectively expressed on tumor cells are potential therapeutic agents with high specificity. Recently, the efficacy of the antibody approach to cancer therapy has been revolutionized by such advances as the identification of novel antigens which are homogeneously expressed in the tumor but have low expression in normal vital tissues, and by genetic engineering techniques to produce tailor-made targeting vehicles with reduced immunogenicity and improved effector functions. An impressive example is given by the HER-2/neu-specific humanized antibody Herceptin, which enhanced the chemosensitivity of tumor cells and lengthened remission times in patients with refractory metastatic breast cancer [22]. HER-2/neu is also over-expressed in a large proportion of NSCLCs, but is rarely associated with SCLC. In addition to the use of intact antibodies to recruit host effector cells and complement components or to inhibit growth factor signaling, rationally engineered full-size antibodies and single-chain Fv fragments have also been used in imaging and therapy studies to deliver radioisotopes, anti-tumor effector molecules, or immune response modifiers to solid tumor cells [23–26]. Single-chain Fv fragments are small molecules of about 25–30 kDa with improved tumor penetration ability. They can be genetically fused with effector proteins or chemically coupled with chemotherapeutic agents. Recent progress in phage display technology has expanded our ability to select human antibodies and construct an array of derivatives with desirable clinical properties. This technique also provides a powerful tool to search for novel target molecules selectively expressed on the surface of tumor cells.

The therapeutic efficacy of antibodies and antibody-effector conjugates not only depends on their tumor-targeting potential, but also on the intrinsic properties of the antigen. For example, it is mandatory that the antigen is differentially expressed between tumors and normal tissues, that it is uniformly expressed by the tumor cell population, and that it is not shed from the cell surface or down-modulated upon antibody binding. Moreover, if the target site of a conjugate is located intracellularly, the antigen-antibody complex must be internalized from the surface by receptor-mediated endocytosis. In an attempt to develop novel antibody-based treatment approaches, there has been a collaborative effort between individual laboratories. The Second and Third International Workshops on Lung Tumor Antigens addressed the characterization and definition of surface molecules expressed on lung cancer cells and recognized by monoclonal antibodies [27, 28]. As a result, the nature and biological function of many of these antigens have been characterized. Many of the antibodies that recognize cell surface antigens associated with SCLC have also been investigated in vivo, e.g., in models of SCLC xenografts. These models are useful tools to assess the tumor localization and anti-tumor activity of antibodies and antibody-

Table 1. Potential targets on SCLC cells for antibody-based diagnosis and therapy.

Antigen	Biological properties	References for clinical trials in cancer patients
NCAM (CD56, NKH01)	homophilic cell adhesion molecule of 145 kDa	32, 37
EGP-2 (Ep-CAM, EGP-40, KSA, GA733-2)	homophilic cell adhesion molecule of 40 kDa	42, 43
Lewis Y	blood-group-related carbohydrate with developmentally regulated expression	51, 53
Gangliosides	glycosphingolipids involved in cell interaction with extracellular matrix	59–62

based immunoconjugates under complex physiological conditions. Since most of the antibodies tested do not cross-react with murine tissues, however, their clinical usefulness cannot be fully assessed in animal models. For example, antibodies recognizing CD24 and CD56 (neural cell adhesion molecule, NCAM), antigens also expressed on human hematopoietic cells but not their murine counterparts, efficiently localized to SCLC xenografts in mice [29, 30]. The antibodies, however, failed to detect tumors in patients, where they mainly localized to recticuloendothelial tissues [31, 32]. Based on these observations and the finding that most of the tumor-associated antigens are also expressed in normal vital tissues, only a few antigens remain that may serve as potential targets for antibody-based diagnosis and therapy of SCLC (table 1). The preclinical and clinical studies describing the use of these candidate antigens are reviewed below.

Neural cell adhesion molecule

NCAM, also known as CD56 or NKH01, was designated as a cluster-1 antigen on lung tumor cells [28]. NCAM is a homophilic cell adhesion molecule of 145 kDa abundantly expressed in virtually all SCLC cell lines and cells derived from tumor specimens [33, 34]. In normal tissues, NCAM expression is prominent in nerves, endocrine cells, natural killer (NK) cells and striated and cardiac muscle [35]. Monoclonal antibodies with specificity for NCAM were successfully used for imaging and therapy of SCLC xenografts growing in athymic mice [30, 36]. In a recent phase I study, 21 patients with relapsed or refractory SCLC were treated with the monoclonal antibody N901 which was conjugated with the protein toxin blocked ricin (bR) [37]. Patients received the immunotoxin N901-bR daily for 7 days by continuous infusion. Dose-limiting toxicity was vascular leak syndrome, whereas neuropathy was not observed. Most patients developed either human anti-mouse antibodies (HAMAs) or antibodies against the ricin moiety of the immunotoxin (HARA). One patient achieved a partial response which persisted for 3 months, 6 patients had stable disease. Although this result is encouraging, in view of the finding of Ornadel et al. [32] that NCAM antibodies do not efficiently localize to tumors in patients, the mechanisms underlying the clinical efficacy of N901-bR remain unclear.

Epithelial glycoprotein-2 (EGP-2)

EGP-2, synonymously named Ep-CAM, EGP40, KSA, or GA733-2, was designated as a cluster-2 antigen on lung tumor cells [28]. EGP-2 was first cloned from a lung adenocarcinoma cell line by Strnad et al. [38]. It is a homophilic cell adhesion molecule of 40 kDa abundantly expressed on a variety of epithelial tumor cells, including a major fraction of SCLC [39]. Expression of EGP-2 is dynamically regulated during cell growth and differentiation, and recent investigation has identified its role in tissue invasion and its up-regulation in cells under growth-promoting conditions [40, 41]. The latter finding might explain the different levels of EGP-2 expression, which is high in malignant and low in normal epithelial cells. In a 7-year follow-up study in patients with advanced colorectal cancer, the mouse monoclonal antibody 17-1A (Panorex) produced significant anti-tumor effects [42], and a humanized recombinant variant is currently under clinical investigation in patients with refractory prostate, lung, or colon cancer. The mouse monoclonal antibody MOC31 was used for imaging studies in patients with SCLC [43], and is currently being re-engineered by our group in a collaborative effort to produce humanized scFv variants with high antigen-binding affinity [44].

Lewis Y antigen

A blood-group-related carbohydrate, the Y difucosylated hapten (Lewis Y antigen), also designated as a cluster-6 antigen on lung tumor cells [28], is associated with 60–90% of human tumors of epithelial origin, including breast, colon, and gastric cancer, and SCLC [45–48]. Its level of expression correlates with survival in patients with lung cancer [49]. The Lewis Y antigen is expressed in a stage-specific manner during embryonic development of the human lung, and its emergence on

lung tumor cells appears to be the result of retrodifferentiation of cells to the stages of embryonic lung cells [50]. Despite high lytic activity in the serum of patients with SCLC, the mouse monoclonal antibody ABL364 could not produce clinical responses [51]. Cytotoxic immunoconjugates targeting the Lewis Y antigen have also been tested in preclinical and clinical studies [52, 53]. The immunotoxin LMB-1, composed of the monoclonal antibody B3 chemically linked to a genetically engineered form of *Pseudomonas* Exotoxin A, was used in a phase I study of 38 patients with solid tumors who failed conventional therapy [53]. Objective anti-tumor activity was observed in 5 patients, 18 had stable disease, 15 progressed. A complete remission was observed in a patient with metastatic breast cancer. A greater than 75% tumor reduction and resolution of all clinical symptoms lasting for more than 6 months was observed in a colon cancer patient with extensive retroperitoneal and cervical metastasis. Results from clinical trials with LMB-7, an immunotoxin which employs a humanized scFv version of B3 [54], and with LMB-9, a scFv stabilized by an interchain disulfide instead of a peptide linker, are awaited. These studies will reveal whether the impressive anti-tumor activity of improved second-generation immunotoxins in vitro and in animal models translates into therapeutic efficacy in patients.

Gangliosides

Gangliosides are glycosphingolipids, composed of a ceramide portion that anchors it into the plasma membrane and a carbohydrate moiety oriented to the extracellular space. Due to differences in the carbohydrate residues, many distinct gangliosides have high levels of expression particularly in neuroendocrine tumors such as melanoma and SCLC, but low levels of expression in normal tissues. Gangliosides are reported to be involved in the attachment of tumor cells to extracellular matrix proteins [55]. Based on their differential expression in tumors, the gangliosides GD2, GD3, and GM2 represent the most promising targets of antibody therapy [56–58]. A mouse-human chimeric antibody targeting GM2 was reported to induce effector-cell-mediated lysis of SCLC cells in vitro [58], and antibodies targeting GD2 demonstrated tumor-localizing and therapeutic potential in patients with melanoma and neuroblastoma [59, 60]. In a recent pilot study, the mouse monoclonal antibody 3F8 was used to target residual disease in SCLC patients [61]. Twelve patients were enrolled in this trial and 10 received intravenous 3F8 labeled with ^{131}I. Five patients had recurrent or progressive disease after chemotherapy, 7 patients were subjected to diagnosis prior to the initiation of chemotherapy. Radionuclide scans demonstrated localization to all known sites of disease, apart from small brain metastases in 1 patient.

Anti-GD3 antibodies were also used to raise anti-idiotypic antibodies against GD3 in mice. In recent clinical trials, the anti-idiotypic antibody BEC2 was used to immunize patients with melanoma and SCLC [62]. Fifteen patients, 8 of whom presented with extensive disease, were enrolled in the study. BEC2 was administered intradermaly together with bacille Calmette-Guérin as an immune adjuvant. All patients developed anti-BEC2 antibodies. Five of 13 patients evaluable for serologic response developed anti-GD3 IgG antibodies. For these patients, survival from the time of initial diagnosis was significantly improved compared to the median survival of historical control groups. In view of these encouraging results, further clinical studies have been initiated to evaluate the potential of anti-ganglioside and anti-idiotypic ganglioside therapy in SCLC.

Targeting growth factors and growth factor receptors

An autocrine growth loop in cancer was first described as a potential growth pathway for tumor cells more than 17 years ago [63]. A variety of neuroendocrine peptides such as the bombesin-like peptide gastrin-releasing peptide (GRP), and insulin-like growth factor-I (IGF-1) fit the definition of autocrine growth factors for SCLC. With the aim of inhibiting the growth of SCLC cells, these growth factors and their receptors have been evaluated as potential targets for various antagonists including antibodies and for antisense therapeutics.

Neuroendocrine growth factors

SCLC is a neuroendocrine tumor which, according to the degree of neuroendocrine differentiation, is divided into a classic and a variant phenotype [64]. Classic SCLC accounts for 70% of all SCLCs. These tumor cells are characterized by dense-core neurosecretory granules and the production of high levels of neuroendocrine growth factors and peptide hormones that act as autocrine growth factors, such as dopa decarboxylase, neuron-specific enolase, and bombesin-like peptides. The bombesin-like GRP was initially cloned from lung carcinoid tumors and SCLC cell lines. It is a 27-amino-acid peptide produced from a large precursor protein of 145 amino acids that binds to its receptor with high affinity in the nanomolar range [65, 66] stimulating the growth of SCLC cells by elevating cytosolic Ca^{2+} and activating protein kinase C [67–69]. Synthetic GRP receptor antagonists and antibodies neutralizing secreted GRP have been used in a variety of preclinical studies to interrupt the autocrine and paracrine growth factor loops, and to inhibit the prolif-

eration and clonogenicity of SCLC cells in vitro and in vivo. The broad-spectrum receptor antagonist substance P was identified as a potent analog in inhibiting bombesin-like peptide receptor binding in vitro [70]. The results of a phase II trial with the GRP-neutralizing monoclonal antibody 2A11 were published recently [71]. Patients with relapsed or refractory SCLC received 12 doses of 2A11 over a 4-week period. Twelve of 13 patients were eligible: 1 had a complete response to treatment, 4 achieved stable disease, and 7 showed progression. The responding patient received another cycle of treatment with 2A11 and remained without evidence of progression for 5 months. Since the growth of SCLC cells depends on multiple growth factors, more studies are necessary to determine the therapeutic potential of bombesin-like peptide inhibitors in patients with SCLC.

Insulin-like growth-factor-I

IGF-I is a 70-amino-acid growth factor found at elevated levels in more than 90% of SCLC tumors [72–74]. It is involved in mitogenic signaling by binding to tyrosine kinase receptors causing phosphorylation of protein substrates and cell growth [75]. Specifically, the apoptosis-inhibiting pathway of IGF-I seems to route through Ras, PI-3 kinase, and the serine-threonine kinase Akt/protein kinase B [76, 77], ultimately impacting on Bad, a key modulator of Bcl-2 [78]. The IGF-I receptor is expressed in many human tissues and tumors including NSCLC and SCLC [74]. It is regulated by p53 and functions as an autocrine growth factor implicated in the apoptotic response of cells [79]. Analogously to the GRP autocrine growth loop, the IGF-I pathway can also be inhibited using receptor antagonists or ligands neutralizing the growth factor. Antibodies that block the specific binding of IGF-I to its receptor, such as the monoclonal antibody αIR-3, are able to inhibit the growth of SCLC and NSCLC cells in vitro and in vivo [80].

IGF-I signaling in cells is inhibited by antisense oligodeoxynucleotides (ODNs) that repress expression of the IGF-I receptor. This has been demonstrated by Resnicoff et al. [81] who used antisense ODNs to reduce the number of IGF-I receptors on melanoma cells to inhibit their tumorigenesis in vivo. The antisense approach to inhibition of cancer-related gene expression has become particularly interesting since many of the initial limitations associated with antisense ODNs, such as limited stability, poor specificity, unpredictable targeting, and undesired non-antisense effects, have now been solved by chemical modifications [82, 83]. In addition, improvements in the manufacturing process have dramatically reduced the overall costs of ODN synthesis [84]. Antisense ODNs are fast, simple, and cost-effective tools available for validating new therapeutic targets, commonly the first step in modern drug development. The ability of these compounds to inhibit the expression of genes involved in cell growth and apoptosis regulation in a sequence-specific manner has been demonstrated in various preclinical and clinical studies [85–87].

Targeting the expression of drug transport proteins

The issue of drug resistance in SCLC has been addressed extensively and genetic abnormalities that might contribute to the resistance phenotype, such as over-expression of genes coding for drug efflux pumps located in the plasma membrane, have been identified [88, 89]. The role of drug transporters in clinical drug resistance, however, remains unclear. Whereas the absence of MDR1 gene expression during chemotherapy of SCLC indicated a favorable prognosis in one study [90], in another study, no correlation between MDR1 gene expression in SCLC and NSCLC cells and chemosensitivity and clinical response to therapy was detected [91]. Recently, novel second-generation antisense ODNs targeting the MDR1 mRNA were used to down-regulate P-glycoprotein expression and increase drug uptake into cells in vitro [92]. To what extent this approach can help overcome clinical drug resistance in SCLC and other refractory tumors remains to be determined.

Targeting the expression of oncogenes and apoptosis inhibitors

Considerable evidence has accumulated that cancer has a genetic origin based on the occurrence of mutations in families of genes implicated in DNA repair, growth control, and apoptosis [93–95]. This provides researchers with the opportunity to specifically target genetic lesions and abnormal gene products by therapeutic interventions. The most extensively investigated and advanced approaches to gene therapy of cancer are: (i) the use of antisense ODNs to inhibit the expression of oncogenes; (ii) replacement of inactivated or lost tumor suppressor genes; (iii) ex vivo transfection of autologous tumor cells with cytokine genes and their use as vaccines to stimulate an anti-tumor immune response.

The bcl-2 family of apoptosis inhibitors

Current research has provided evidence that the inability of tumor cells to undergo programmed cell death or apoptosis in response to cytotoxic damage is a critical

determinant of drug resistance [96, 97]. The search for gene products involved in the regulation of cell growth and apoptosis has identified numerous proteins, and the list continues to grow. In particular, much interest has been devoted to members of the bcl-2 gene family of apoptosis regulators, because of their contribution to oncogenesis and drug resistance. This family of structurally related proteins includes members that exert opposing functions in apoptosis control. The Bcl-2 protein functions in normal and neoplastic cells to inhibit or delay apoptosis induced by a variety of endogenous and exogenous stimuli [98, 99]. The related Bcl-xL protein also acts as a negative regulator of apoptosis and protects cells from cell death where Bcl-2 is ineffective [100]. Over-expression of bcl-2 has been found in 70–90% of SCLC cell lines and tumor specimens [101–103]. We tested a number of first-generation phosphorothioate ODNs hybridizing to different regions of the bcl-2 mRNA [85]. These experiments revealed a sequence in the coding region as the most effective target site for antisense-mediated down-regulation of bcl-2 expression and induction of apoptosis in SCLC cells. Moreover, repression of the bcl-2 gene using this antisense ODN (2009) was shown to sensitize SCLC cells to chemotherapy, resulting in a synergistic cytotoxic effect [104]. In a recent phase I trial, the phosphorothioate antisense ODN G3139, which targets the translation initiation site of the bcl-2 mRNA, was administered as a 14-day continuous subcutaneous infusion in nine patients with relapsing non-Hodgkin lymphoma [105]. A reduction in tumor size was achieved in two patients, and in another two patients, the number of circulating lymphoma cells decreased during treatment. The dose-limiting toxicity of G3139 was related to the unspecific protein-binding activity of the phosphorothioate backbone [106]. The use of second-generation bcl-2 antisense ODNs with improved biological and pharmacological properties is under consideration in patients with refractory tumors.

In addition to bcl-2, the anti-apoptotic bcl-xL gene is also expressed in a number of human tumors of different histological origin including SCLC [73, 107, 108]. Since expression of bcl-xL also substantially contributes to resistance of tumor cells to a broad range of anti-cancer agents, it represents a further promising target for antisense therapeutics. That bcl-xL antisense ODNs can indeed induce apoptosis in SCLC cells is shown by the electron micrographs in figure 2. The role of bcl-xL antisense ODNs in cancer therapy and other proliferative diseases is currently under preclinical evaluation by several investigators.

The myc family of oncogenes

In addition to the bcl-2 family members of apoptosis inhibitors, the myc family of oncogenes, which is transcriptionally deregulated or amplified in many human tumor cells including a significant proportion of SCLC cells [109–111] also represents an attractive target for gene repression approaches. The Myc phosphoproteins are transcription factors of the bHLH-zip family and there is substantial evidence for their role in the control of cell proliferation. In certain circumstances, however, Myc also promotes apoptosis [112–114]. Although the precise mechanism for this function remains obscure, Myc-induced apoptosis is efficiently suppressed by Bcl-2, which acts as a downstream inhibitor [115], and by survival signals, such as IGF-I or interleukin (IL)-3 [76, 116]. The higher level of Myc found in pretreated patients compared to patients prior to chemotherapy [117, 118] implies that it might be a negative regulator of apoptosis in SCLC cells. This finding is consistent with studies demonstrating poor response to chemotherapy and worse survival in patients with myc gene over-expression [119]. Antisense ODNs targeting the c-myc mRNA were used to inhibit the growth of NSCLC and to induce apoptosis in leukemia and melanoma cells in vitro and in vivo [120–122].

Cyclins

The passage of a cell through the cell cycle is controlled by cytoplasmic proteins, the most critical being cyclins and cyclin-dependent kinases (cdks), and alterations in cyclin expression provide the earliest examples of cell cycle regulators acting as oncogenes [123]. Cyclin D expression begins in early G_1, when quiescent cells are stimulated to re-enter the cell cycle, and cyclin D expression remains at high levels as long as mitogens and proliferative signals are present. Cyclin D1 is the product of the bcl-1 gene, located near the translocation breakpoint (11;14) in B cell lymphomas. Cyclin D1 together with its cdk partner is responsible for transition to the S (DNA synthesis) phase by phosphorylating the product of the retinoblastoma susceptibility gene (Rb), which then releases transcription factors important in the initiation of DNA replication [124]. Inhibition of cyclin D1 blocks the cell cycle in G_1, demonstrating the necessity of cyclin D for cell cycle control [125, 126]. Furthermore, reinforced expression of cyclin D1 can mimic the effects of growth factors in activating the cyclin E-Cdk2 complexes and promoting S phase entry [127]. These results suggest that cyclin D1 might be important for tumorigenesis. Further evidence for cyclin D1 as an oncogene was provided by experiments in which cyclin D1 over-expression transformed BRK cells with a defective adenovirus E1A protein [128] or rat fibroblasts with an activated Ha-ras gene [129]. Cyclin D1 over-expression may, however, be an early and purely proliferative event that eventually becomes oncogenic as further genetic aberrations emerge. Over-expression of cyclin D1 has also been identified as a frequent and early event during the development of NSCLC, where it is associated with a poorly differentiated histology and, surprisingly, a reduction in local relapse rate [130, 131]. Although overexpres-

sion of cyclin D1 is a key abnormality in lung carcinogenesis, it seems to be a less frequent event in SCLC, where its prognostic and therapeutic significance have not yet been addressed. Based on its frequent alteration and role in cell cycle control, cyclin D1 may serve as a potential target for gene inhibition approaches in early lung cancer. As shown by Schrump et al. [132], down-regulation of cyclin D1 expression using an antisense approach could reduce the proliferation and tumorigenicity of murine lung cancer cells.

Prostate tumor-inducing gene 1 (PTI-1)

Expression cloning and differential RNA display experiments revealed a novel potential oncogene of 2123 bp, PTI-1, which is expressed in prostate, breast, colon, and SCLC cells [133, 134]. In a recent study, Su et al. [135] demonstrated that stable expression of the nearly full-length 1.9-kb PTI-1 gene in fibroblasts induced an aggressive phenotype with increased tumorigenicity in athymic mice. Blocking of PTI-1 expression with an antisense gene construct could revert the transformation of the cells and reduced their tumorigenic potential. Further studies are necessary to reveal whether PTI-1 indeed represents a potential target for antisense therapeutics in SCLC and other cancer diseases.

Gene therapy

Replacement of tumor suppressor genes

Especially in tumors with a high propensity to metastatic spread such as SCLC, there are severe limitations in the efficiency of gene transfer by currently available vectors and gene transfer systems. Preclinical and clinical studies in other tumor types including NSCLC, however, have suggested that current delivery strategies may have therapeutic potential in defined clinical settings.

In SCLC cells, a number of genetic lesions have been reported, including the well-characterized tumor suppressor gene p53 and Rb, which are mutated or lost in 90% and 70% of cases, respectively. Wild-type p53 is a sequence-specific transcription factor whose expression

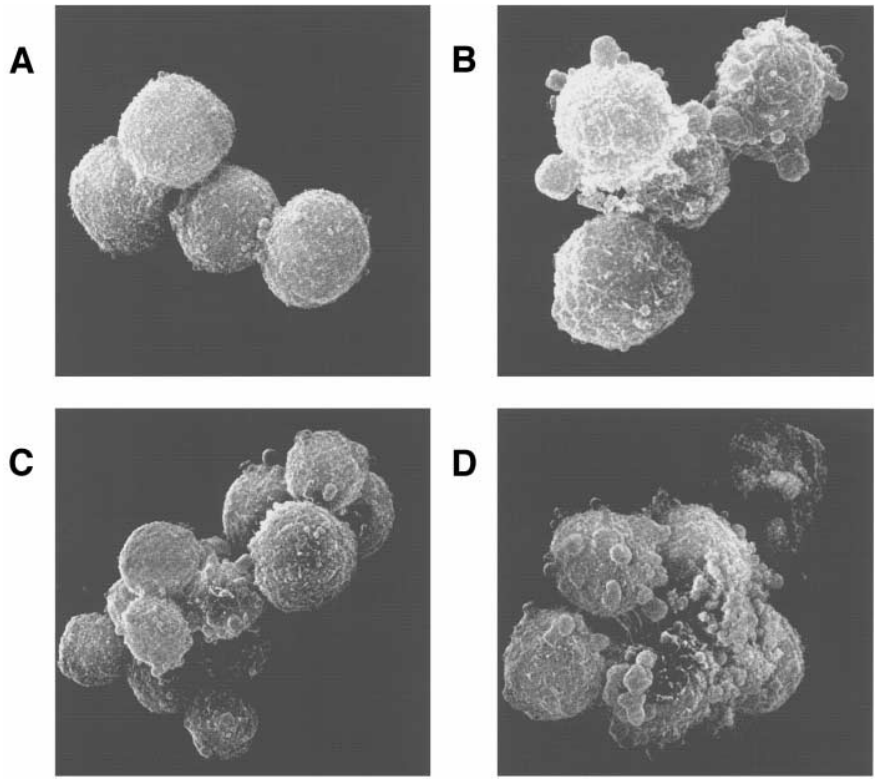

Figure 2. Electron mircographs of SW2 SCLC cells 96 h after treatment with bcl-2 or bcl-xL antisense oligodeoxynucleotides (ODNs): untreated cells (*A*), cells treated with a bcl-2 antisense ODN (*B*), cells treated with a mismatch control ODN (*C*), cells treated with a bcl-xL antisense ODN (*D*). As shown by the extensive membrane blebbing, cell death after treatment with the antisense ODNs was apoptotic by nature. (Prof. P. Grosscurth, Institute of Anatomy, University of Zürich, with permission).

can suppress the progression of cells from G_1 into S phase [95, 136]. It is also required for the G_1 arrest of cells in response to DNA damage, presumably to allow cells to repair damaged templates prior to replication [137, 138]. Substantial evidence suggests that a major part of p53-mediated growth arrest evolves through induction of the cdk inhibitor p21WAF1 (p21) [139]. In contrast, the mechanism by which p53 promotes apoptosis is less clear. Examples of p53-regulated targets are proteins involved in the apoptotic response, such as the Bcl-2 antagonist Bax [140, 141] and the IGF-I receptor [79], and proteins regulating angiogenesis [142, 143].

In its hypophosphorylated state, the Rb protein causes cell cycle arrest in the G_0/G_1 phase, in part by inhibiting the function of transcription factors such as E2F [144, 145]. Inappropriate passage of cells through the G_1 checkpoint may cause them to replicate damaged DNA in the S phase before DNA repair can be completed in G_1, and to ignore signals inducing differentiation or cell death [146].

In view of these findings, p53 and Rb gene replacement might be of therapeutic benefit for many tumor types. Gene replacement with retroviral or adenoviral vectors harboring wt-p53 was shown to restore the apoptotic response of NSCLC cells in preclinical studies. In a recent phase I study with 21 patients presenting with NSCLC, local injection of the p53 adenoviral vector alone or in combination with cisplatin lengthened the time to disease progression and produced a bystander effect, possibly due to the anti-angiogenic effect of p53 [147]. The p53 tumor suppressor gene transactivates the cdk inhibitor gene p21, which is an important mediator of cell proliferation and differentiation [148]. In cell cycle regulation, p21 is a dual inhibitor of cdks and proliferating cell nuclear antigen, both of which are required for entering and progressing through the S phase of the cell cycle [149, 150]. Cells over-expressing p21 accumulate in the G_1 phase and p21 null cells are impaired in their ability to undergo G_1 arrest following DNA damage [149, 151]. In a recent preclinical study, p21 gene replacement using an adenoviral vector inhibited the growth of NSCLC cells in vitro and in a xenograft model by inducing G_0/G_1 arrest [152]. Since the p53 and p21 tumor suppressor genes are frequently mutated or lost in SCLC cells, gene replacement approaches might be interesting treatment options for patients with limited-stage SCLC. Replacement of the Rb gene using adenoviral vectors inhibits the proliferation of and induces G_1 arrest in Rb-altered human tumor cell lines including SCLC in vitro and in vivo [153, 154].

Vaccination gene therapy and immune activation

Engineering of tumor cells with cytokine genes to enhance their immunogenicity has been the subject of extensive investigation in recent years [155, 156]. IL-2 has been the most commonly used cytokine in these studies, which mediates its immune stimulatory effect by binding to a specific receptor constitutively expressed on T and NK cells [157, 158]. A clinical study suggested a possible effect of exogenous IL-2 in patients with SCLC [159], and an approach to genetically engineer autologous SCLC cells from patients with limited-stage SCLC to produce IL-2 has been proposed [160]. As shown by Meazza et al. [161], transfection of the IL-2 gene into SCLC cells reduced their tumorigenicity in nude mice, due to the recruitment and activation of macrophages, granulocytes, and NK cells at the tumor site. The idea underlying this approach is to prevent anergy of immune competent cells and to activate a systemic immune response by creating an environment of high doses of interleukins at the tumor site. A variety of epithelial tumor cells, including SCLC and NSCLC cells, however, up-regulate expression of the Fas-ligand (CD95L) on their surface, thereby creating an immune-privileged environment almost inaccessible for cytotoxic T cells [162]. Further studies are needed to assess whether the rationale of the cytokine gene therapy approach will translate into clinical efficacy.

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