GENES : A Novel Treatment for SCLC

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Abstract

Small Cell Lung Cancer (SCLC) is a highly aggressive cancer with widespread metastasis at presentation. SCLC patients have a poor prognosis with very high mortality when treated by standard treatment designs of chemotherapy, radiotherapy and occasionally surgery, and novel treatment regimes for this malignancy are therefore in great need. As cancers are basically abnormalities in cell growth mediated by altered genetic material, gene therapy constitutes a promising alternative fourth treatment modality for SCLC either in combination with conventional treatment modalities or alone. Targeted gene therapy in the form of transcriptionally targeted gene therapy, tumor suppressor genes and suicide genes is being investigated for SCLC.

1 Introduction

Lung cancer persists as the leading cause of cancer related deaths worldwide (1.35 million new cases per year and 1.18 million deaths)[1]. Based on the histological appearance of cancer cells, lung cancers are divided into two broad classes, Non Small Cell Lung Cancer and Small Cell Lung Carcinoma (SCLC). Though SCLC causes only 15-20% of lung cancers, it is responsible for 50% of lung cancer deaths. SCLC separates from other histological types of lung cancer by a distinct neuroendocrine phenotype, aggressive progression with widespread metastases at presentation and initial high sensitivity to chemo and radio-therapy[2,3]. Symptoms of SCLC usually include a persistent cough, breathlessness, chest pain, weight loss and production of phlegm. There can also be rather vague symptoms such as weight loss and general tiredness. There are no specific serum biomarkers for early detection of SCLC. Currently available serum biomarkers in SCLC improve diagnostic efficiency in the detection of tumor progression in lung cancer and may serve as potential therapeutic targets. E.g. neuroendocrine markers like chromogranin A (CgA), pro-gastrin releasing peptide (ProGRP/ synatophysin) and neuron-specific enolase (NSE; an γ-γ isoform of the ubiquitous enolase enzyme); cytokeratin 19 marker CYFRA 21-1 and cytokines, IL-2 and IL-12[2]. Most of the patients have large tumors, lymph node infiltration and widespread dissemination at the time of detection, leading to its poor prognosis despite standard modes of treatment (median survival rate of only 20 weeks). Hence, there is a great demand for new therapies to supplement or replace the available treatment[1,4-6]. As altered genetic material is the basic molecular pathology in all cancers, gene therapy constitutes a promising strategy and relies on the principle of introducing exogenous DNA to kill cancer cells. Gene therapy is already approved for treatment of Head and Neck cancer (HNC) in China[7]. Hence, though in experimental stage at present, it seems to be a plausible option to be available shortly as a significant therapy for SCLC[8,9].

2. Cytogenetics in SCLC

SCLC results from DNA damage due to carcinogens (e.g. smoking) or spontaneously during DNA replication[10-13]. There are 10-20 errors in each cancer cell, which are self amplified due to failure of error correction and clonal evolution[14-19]. Almost 100% cells show chromosomal loss, mainly in short arms of chromosomes 3,17 and long arms of chromosomes 5,13; leading to inactivation of tumor suppressor genes(TSG) e.g. p53 genes[20,21]. There is amplification of oncogenes e.g. MYC family genes[22-24]. The specific genes affected in SCLC and the proteins they express are enlisted in Table 1.

3. Gene Therapy

Gene therapy is a technique for correcting defective genes responsible for disease development. Several approaches are used for correcting faulty genes[25].

- A normal gene may be inserted into a nonspecific location within the genome to replace a nonfunctional gene. This approach is most common e.g. insertion of tumor-suppressor candidate 2 (FUSI/TUSC2) in SCLC. A vector or a carrier molecule is used to deliver the therapeutic gene to the target cells. The generation of a functional protein product from the therapeutic gene restores the target cell to a normal state[25].
- An abnormal gene could be swapped for a normal gene through homologous recombination e.g. in thalassemia.
- The abnormal gene could be repaired through selective reverse mutation, which returns the gene to its normal function e.g. in combined immunodeficiency.
- The regulation (the degree to which a gene is turned on or off) of a particular gene could be altered e.g. use of transcriptionally targeted hASH1 promoter (Oncogene) in SCLC[25]

More than half of all ongoing clinical trials for gene therapy aim at cancer[26]. Cancer gene therapy relies on the principle of introducing exogenous DNA into malignant cells causing them to die. Gene therapy possesses unique possibilities for the targeting of tumor tissue not obtainable by current cancer therapies, such as chemotherapy, where off target toxicity limits the optimal therapeutic dosing of patients[27].

4. Gene Therapy for SCLC

Table 1- Genes and Proteins in Small Cell Lung Carcinoma

Gene	Location on chromosome	Frequency	Abnormality	Class	Protein Expressed	Function of the gene
p53	3p14	70%	Downregulated	TSG	DNA-BP-1	Nuclear role-transcription Cytoplasmic role-regulates cell cycle, cell division, apoptosis
MYC MYCL MYCN c-MYC	1p32 2p23 8q24.1	60-70%	Amplified, Mass in drug resistant relapsed tumor	Oncogene	Transcription Factors	Cell Proliferation, Repression of Growth suppressing genes.
RB1	13q14	>70%	Downregulated	TSG	Retinoblastoma protein	Inhibition and epigenetic inactivation of p16
hTERTgene	5p15	40%	Upregulated	Oncogene	Human telomerase reverse transcriptase	Add telomeric repeats to chromosomal ends to maintain replication
FHIT	3p14.2	60%	Downregulated Epigenetically	TSG	FHIT(Histidine Triad protein	Inhibit cell growth, induce apoptosis
PIK3CA PIK3CB	3q26.3	100%	Downregulated Epigenetically	TSG		Antiproliferation, Apoptosis, altered cell cycle kinetics
RASSF1A	3p21.3		Downregulated Epigenetically	TSG		Stabilize microtubules, cellular adhesion, mobility by stabilizing microtubules

Source: Ref. 20-24.

As SCLC is usually a widely disseminated tumor and is not superficially located, only the targeted gene therapy (where the therapeutic genetic material enters or expresses only in the desired specific target cells) is useful and treatment has to be administered systemically. Targeting of the tumor phenotype by gene therapy in SCLC can be achieved in several ways as shown in Figure 1.

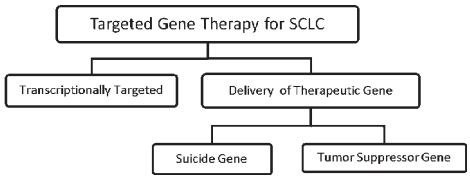

Figure 1 : Modalities of Targeted Gene Therapy for SCLC.
Source: Ref. 27.

4.1 Transcriptionally Targeted Gene Therapy

A cancer-specific promoter is used to specifically control therapeutic gene expression and resulting cytotoxicity in the cancer cells, while avoiding gene expression in normal tissues. Interestingly, the major candidates for this, like the INSMl promoter, the hASHl promoter and the EZH2 promoter, are all involved in the neuroendocrine phenotype of SCLC. The various systems being studied are [27]:

a- MYC family proto-oncogenes - Overexpression of the MYC family proto-oncogenes in SCLC is well known. When MYC heterodimerizes with the protein Myc-associated factor X (MAX) (Figure 2)

b- The resulting active transcription complex recognizes MYC-MAX response elements (MMREs) with high affinity, causing the transcription of downstream genes. By inserting multiple MMREs into highly active nonspecific promoters, therapeutic gene expression and efficacy in SCLC cell lines and xenografts can be achieved. The MYC proteins are expressed at low levels in normal tissues and transactivate genes involved in cell proliferation, differentiation and apoptosis[27,28].
Myc (cMyc) codes for a protein that binds to the DNA of other genes. When Myc is mutated, or overexpressed, the protein does not bind correctly, and often causes cancer.

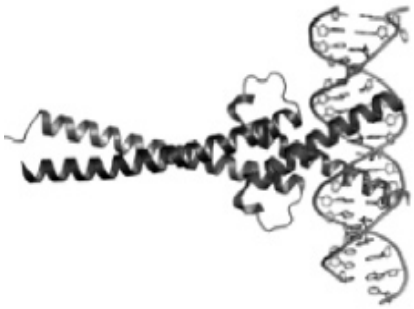

Figure 2. Structure of the c-Myc (red) in complex with Max (blue) and DNA. Both proteins are binding the major groove of the DNA by forming a fork-like structure.

c- Promoter of the hTERT gene - Expression of a therapeutic gene from the hTERT promoter can inhibit cell growth in a SCLC cell line. A construct combining a and b shows increased activity than either construct alone.

d- The promoter from the gastrin-releasing peptide (GRP), a clinical tumor and serum marker in SCLC, was capable of inducing significant cytotoxicity in both SCLC cell lines and xenografts. GRP is known to be expressed at low levels in the nervous system, GI tract and lungs which reduces specificity of its use.

e- The neuron-specific enolase promoter for SCLC gene therapy displayed high specificity, but only a moderate promoter activity causing equally modest therapeutic gene expression and effect.

f- INSMl promoter - The insulinoma-associated 1 (INSM1) gene is highly re-activated in neuroendocrine tumors, including SCLC and expresses a therapeutic gene to cause SCLC specific cell

death in vitro and in vivo[28].

g- The hASHl promoter - The Achaete-Scute Homolog 1 (ASCL1) encoding a transcription factor transiently expressed during early development of neural and neuroendocrine progenitor cells, is also re-expressed in SCLC and promoter region regulating expression of a therapeutic gene was found to mediate SCLC specific cell death in vitro[28].

h- EZH2 promoter - The Enhancer of Zeste Homolog 2 (EZH2) gene is a member of the Polycomb group of proteins important for maintaining the silenced state of homeotic genes in the adult and is associated with high proliferation. EZH2 promoter displayed very high activity in SCLC cell lines, but not in other cell lines and was sufficiently active to mediate SCLC cell death in vitro[28].

i- The chimeric promoter consisting of the hASHl and EZH2 promoter add to the specificity and activity of promoter constructs and is superior to either promoter alone[27].

j- Insertion of cytomegalovirus (CMV) and simian virus (SV)40 enhancer sequences increase the activity of the promoters but decrease the specificity of the constructs[27].

4.1.1 Disadvantages

1. The activity of promoter sequences derived from genes associated with a specific phenotype, in this case the neuroendocrine phenotype, might be compromised as a result of tumor heterogeneity (one tumor can have many different types of cells because tumor cells can have genes and proteins that are very different from one another and thus can grow at different rates) and resistance. A decrease in transcriptional activity of the promoter regions will cause treatment outcomes to be limited accordingly.
2. Incomplete in vivo delivery may require even higher activation of gene expression in the tumor cells of interest than can be accomplished from the endogenous promoter candidates identified to date. Therefore, it might be interesting to re-analyze and recluster existing SCLC gene-expression data in search of short regulatory elements that are consistently present in the upstream regions of SCLC up- and down-regulated genes. By combining the most promising positive and negative regulatory elements obtained from this analysis, superior synthetic promoters might evolve[27].

4.2 Potential Therapeutic Genes for SCLC

In comparison to transcriptional targeting, less focus has been on the identification of suitable therapeutic genes for SCLC, with only a few genes tested. Figure 3 summarizes the possible design of a gene therapeutic drug for SCLC. Two major and very different categories of therapeutic genes, suicide genes (SGs) and TSGs, are currently used (FIGURE 3 B & C).

4.2.1 Suicide Genes

In SG therapy, the introduced therapeutic gene encodes an enzyme capable of transforming a nontoxic, systemically applied prodrug into a cell poison. The universal cytotoxic mechanism of SG therapy, comparable to chemotherapy, obligates a high level of targeting to accomplish the exclusive activation of prodrug in the malignant cells.

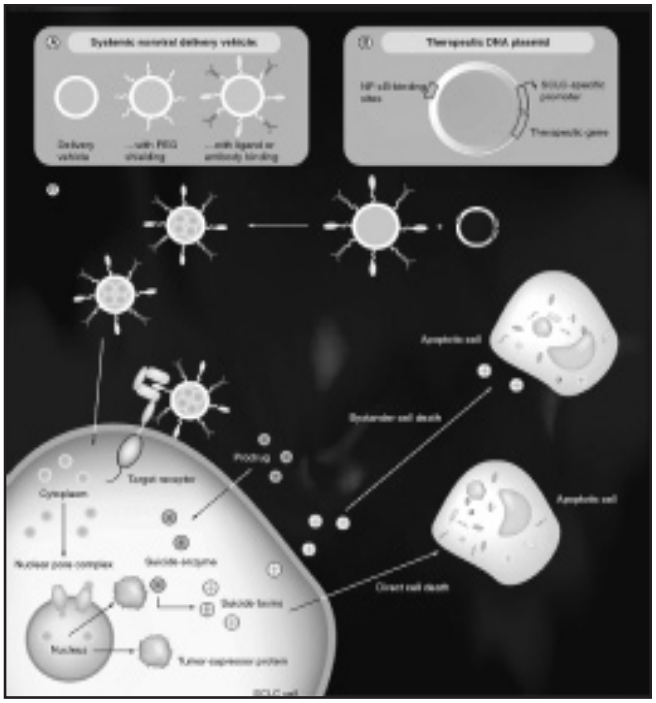

Figure 3. Summary of possible gene therapeutic designs for small-cell lung cancer. (A) Modifications of nonviral delivery vehicles for SCLC gene therapy Modifications of liposome-based delivery vehicle with either poly-ethylene-glycol (PEG) or receptor-binding ligand or antibody. (B) Therapeutic DNA plasmid constructs for SCLC gene therapy consisting of a SCLC-specific promoter, therapeutic gene and NF-κB-binding site. The SCLC-specific promoter mediates specific expression of the therapeutic gene in SCLC cells. The NF-κB-binding site is incorporated into the therapeutic plasmid to promote increased nuclear translocation. (C) Overview of SCLC gene therapy showing gene delivery over the cellular and nuclear membrane, therapeutic gene expression and resulting cell death either as a result of direct cytotoxicity (tumor-suppressor genes and suicide genes) or bystander cytotoxicity (suicide genes only). Source: Ref 27.

Table-2 enlists the characteristics of ideal SG system for which research is ongoing.

4.2.1.1 The Bystander Effect (BE): The BE allows the spread of active toxins to nearby cancer cells not initially transfected with SG (FIGURE 3), either by free diffusion of toxic metabolites across the cellular membrane or transport via gap junction intercellular communication(GJIC)[29]. Even though it seems, paradoxically, to allow for the uncontrolled spread of toxins when effort is being put into developing robust targeting strategies, bystander cytotoxicity remains important due to the challenges of transgene delivery (described later). The main factor for a safe but efficient BE is a suitable half-life of the toxins, long enough to enable a solid distribution of the suicide toxins in tumor and associated tissues but short enough to limit the escape of toxins to the systemic circulation by inactivation or clearance of the drug before systemic damage [30].

Some of the SG systems undertrial are enlisted in Table 3.

The first SG system to be described was the herpes simplex virus type 1thymidine kinase (HSVTK) in combination with the prodrug ganciclovir (GCV)[31]. The HSVTK gene phosphorylates GCV, forming GCV monophosphate (GCVMP), while the further steps of phosphorylation creating GCV triphosphate (GCVTP) are mediated by endogenous kinases. The incorporation of GCVTP into DNA during replication causes subsequent replication to be disturbed and blocked [19,20]. Although found effective in animal models and clinical

Table 2. Ideal Characteristics of Suicide Gene, Prodrug and Suicide Toxin

Characteristic	Explanation
Suicide Gene	
Is either not found in the human genome, expression is limited in human cells or the resulting human enzyme analogue is incompatible with activity towards the prodrug	No unspecific activation of prodrug is facilitated
Has high catalytic activity at physiological conditions	Toxin formation even at low prodrug concentrations
Permits full prodrug activation independent of the catalytic activity of endogenous enzymes	Endogenous enzymes could be mutated in tumors. Mutations can be a part of the protected tumor phenotype or can arise as a consequence of the treatment-related selection
Prodrug	
Is nontoxic prior to activation	No toxicity at administration of High (therapeutic) prodrug doses
Is chemically stable under physiological conditions (no intrinsic activity)	No spontaneous conversion of prodrug at administration
Has the pharmacological and pharmacokinetic properties for optimal distribution is cold tumor	High concentrations of prodrug in tumor tissue
Toxin	
Freely able to cross cell membranes independent to GJIC to permit bystander cytotoxicity to nearby cancer cells	Inefficiencies of transduction efficacy of tumor tissue. Bystander effects could be independent to GJIC as this function is downregulated in many cancers
Has the pharmacological and pharmacokinetic properties for optimal distribution is cold tumor including suitable half-life	High concentrations of toxin in tumor tissue, while systemic exposure to the systemic circulation is limited by inactivation or clearance of the toxin
Cell phase unspecific and induces cell death in both proliferating and nonproliferating cancer cells	Only a fraction of tumor cells are actively dividing
GJIC: Gap junction intercellular communication	

Source Ref: 27

trials, the therapeutic efficacy of the HSVTK/GCV system is limited by several factors: the BE is dependent on GJIC between cancer cells, GCV causes off-target toxicity and cytotoxicity of toxins (GCVTPs) is dependent on active cell division (cell phase specific),causing only the proliferating cancer cells to be sensitive to treatment. The latter aspect is another major concern in the development of suitable SG therapeutics, as only a fraction of cells in tumor tissues are actively dividing (TABLE 2).

Of the described SG systems, only HSVTK/GCV and Cytosine deaminase(CD)/5-fluorocytosin(5-FC) have been tested in Phase III clinical trials, but the therapeutic significance has been very modest. Although the Nitroreductase(NTR) gene and the CBI954 prodrug have been tested separately in clinical Phase I trials, no trial has yet been performed combining SG and the prodrug. The lack of therapeutic efficacy of SG therapy in clinical trials is mainly ascribed to the lack of optimal delivery of therapeutic DNA to cancer cells[30,32].

4.2.2 Tumor Suppressors

Tumor-suppressor gene(TSG) therapy is based on the reintroduction of a functional TSG into the cancer cells in order to promote antitumorigenic effects by inducing apoptosis and/ or cell cycle arrest. The attraction of using TSGs for treatment of cancers is that they can directly elicit apoptosis in cancer cells without apparent toxicity to normal cells, making specific targeting of cancer cells unnecessary.

The specific sequence of genetic alterations leading to SCLC is still unclear. However, several genetic and molecular changes have been noted, including loss or inactivation of TSGs e.g. by deletion and translocation of chromosomal regions 3p13-14, 4q32-35, 5q32-35, 8p21-22, 10q25, 13q13-14 and 17p12-13 (loss) and 3q26-29, 5p12-13, 8q23-24 and 19q13.1(gain). The TSGs most frequently altered in SCLC are P53 and RB(Retinoblastoma)[27]. Others are mentioned in Table-1.

a- No therapeutic effect of P53 and RB in transiently transfected SCLC cell lines, was observed, questioning their use in SCLC gene therapy .

b- There is a dominant negative mutant P53, forming inhibitory tetramers with wild-type P53. Since mutant P53 also tends to accumulate in the majority of SCLC cells, this explains the lack of effect of P53 expression in transiently transfected SCLC cells. In such cases, an effective approach observed is to reactivate mutant P53 in the cancer cells, which, due to the high level of the mutant protein, would lead to a massive apoptotic response without affecting normal cells expressing low levels of wild-type P53. P53-dependent reactivation and induction of massive apoptosis (PRIMA-I) is a small molecule reported to be capable of restoring tumor-suppressor function to mutant P53 in various

Table 3. Potential Suicide Gene Systems for Small-Cell Lung Cancer Gene Therapy

ENZYME (origin)	PRODRUG	TOXIN	ADVANTAGES	DISADVANTAGES	Clinical Trial
Thymidine kinase (herpes simplex virus type 1)	GCV	GCVMP (GCVTP)	Low affinity for human TK towards GCV; clinically established prodrug	Cell phase-specific cell death; GJIC-dependent bystander effect; GCV-mediated toxicity; dependent on cellular kinases for full prodrug activation	Phase III Clinical trial
Cytosine deaminase (Saccharomyces cerevisiae or Escherichia coli): also fused with uracil phosphoribosyl transferase (S. Cerevisiae or E. Coli)	5-fluorocytosine 5-fluorouracil	5-fluorouracil 5-fluorouracil MP	Cell phase-nonspecific cell death; bystander effect; no human NTK analogue; clinically established prodrug	Dependent on cellular kinases for full prodrug activation (5-fluorouracil fused to UPRT)	Phase III Clinical trial
Carboxylesterase (human or rabbit)	CPT-11 (irinotecan)	SN-38	Cell phase-nonspecific cell death; bystander effect; direct activation of prodrug; clinically established prodrug	Endogenous carboxylesterase activity	Preclinical study in vivo
Tyrosine hydroxylase (murine)	Pro-etoposide (pro-VP-16)	Etoposide (VP-16)	Cell phase-nonspecific cell death; bystander effect; direct activation of prodrug	Endogenous tyrosine hydroxylase activity; no clinical knowledge on prodrug	Preclinical study in vitro
Nitroreductase (E. Coli)	CB1954 (most commonly used); recently new prodrugs have been developed (e.g., SN23862 and SN27686)	DNA-alkylating agent (different chemical name dependent on prodrug)	Cell phase-nonspecific cell death; bystander effect; low affinity of human NTK towards CB1954 (and new prodrugs)	Dependent on cellular kinases for full prodrug activation (fused with new prodrug analogs); little clinical information on prodrug	Phase I Clinical trial
Carboxypeptidase G2 (Pseudomonas aeruginosa)	CMDA or ZD2767P	DNA-alkylating agent (different chemical name dependent on prodrug)	No human analogue to CPG2; cell phase-nonspecific cell death; bystander effect; direct activation of prodrug	No clinical knowledge of prodrug	Preclinical study in vivo

GCV: Ganciclovir; GCVMP: Ganciclovir monophosphate; GCVTP: Ganciclovir triphosphate; GJIC: Gap junction intercellular communication; MP: Monophosphate; SCLC: Small-cell lung cancer; TK: Tyrosine kinase.

Source Ref: 27

cancer cell lines and, hence, induce cancer cell death in vitro and in vivo. Furthermore, PRIMA-I MET, a methylated and more potent form of PRIMA-I, has been shown to act synergistically with several chemotherapeutic drugs, including cisplatin and 5-FU, to inhibit tumor cell growth. This raises the possibility of combining PRIMA-I with SG systems in SCLC in order to achieve a synergistic effect. Furthermore, synergy between PRIMA-I MET and a novel SCLC TSG might be achieved, since the combination of P53 with other TSGs has demonstrated synergistic tumor suppression in lung cancer cells[31].

c- Loss of heterozygosity (LOH) on chromosome 3p is frequently seen in SCLC (90%), mainly in the region of 3p(14-25), strongly suggesting that the region harbors multiple TSGs involved in the origin or development of SCLC. Several genes have been identified within the aforementioned chromosome region, which could be potential TSGs in SCLC, including fragile histidine triad (FHIT), tumor-suppressor candidate 2 (FUSI/TUSC2) and ras association domain family IA (RASSFIA)[7,33].

6. Current Status of Targeted Gene Therapy for SCLC

As only very few gene strategies have been tested for SCLC, no consensus of the best gene strategy for the malignancy exists. SG therapy is indispensable as cytotoxic BEs can only be achieved with SG therapy, but should be used after proper evaluation of off-target toxicity from released suicide toxins. SG therapy obligates a high level of targeting, and specific targeting of the SCLC tumor phenotype can be achieved by the identification of endogenous promoters specifically and highly active in SCLC[14,15]. Tumor heterogeneity and

resistance necessitates the use of a second generation of promoters, based on the combination of several short regulatory elements in SCLC rather than on single endogenous promoter regions

The use of TSGs is beneficial due to intrinsic targeting of the tumor phenotype and allows the use of very strong and unspecific viral promoters such as the CMV promoter without extensive efforts to develop solid targeting strategies. Further insights into the SCLC phenotype are necessary for the selection of efficient TSG restoration therapies for the malignancy[27].

Since most effective cancer treatments of today are based on combinatorial design, the aim should be to design a gene therapeutic modality combining different gene targets and strategies. Studies involving the combined effect of several TSGs and conventional chemotherapy as well as the combination of SG systems have manifested a high potential, increasing efficacy upto 4-6 fold [29,34]. A combination of efficient TSG restoration, SCLC-targeted SG therapy and P53-reactivating therapy would deliver high therapeutic efficacy while reducing toxicity and disease recurrence.

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