SURGICAL AND GENETIC TREATMENT OF ESOPHAGEAL CANCER

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Abstract: *Esophageal cancer is one of the most aggressive types of digestive cancers and due to the usually late diagnosis and to the peculiar anatomical localization, approximately 70% of the patients are in an advanced locoregional stage of the disease or have distance metastases which are unknown at the time of the examination, as they are in general elderly, debilitated and malnourished, often cachectic. In the case of localized esophageal cancer without locoregional extension or metastases, resection surgery remains the best solution. Gene therapy involves a wide variety of types of treatments which use genetic material for the treatment of cancer. This therapy entailed the development of methods to insert certain genes into the genome of the cancer cells by means of various transport systems or the use of other techniques which enable the cancellation of the expression of the genes involved in carcinogenesis or the use of nucleic acid (either DNA or RNA) to influence protein synthesis. Theoretically it is possible to transform either somatic or germinal cells through gene therapy, and gene therapy may be performed both in vitro and in vivo. The medical world shows particular interest towards the introduction into the therapy of specific anti-tumoral agents (antibodies, inhibitors of growth factor receptors, antagonists of the signal transduction factors, vaccines with cells presenting with antigens) associated or not with chemotherapy.*

Cuvinte cheie: *cancer esofagian, tratament chirurgical, genetic*

Rezumat: *Cancerul de esofag este unul din cele mai agresive tipuri de cancer digestiv şi datorită diagnosticului de regulă tardiv, situaţiei anatomice particulare, aproximativ 70% din pacienţi suferă de o boală avansată locoregional sau cu metastaze la distanţă necunoscute, în momentul descoperirii fiind în general în vârstă, taraţi şi malnutriţi, adesea caşectici. Pentru cancerul esofagian localizat fără extindere loco-regională sau metastaze, chirurgia de rezecţie rămâne soluţia cea mai bună. Terapia genică presupune o largă varietate de tipuri de tratament care utilizează materialul genetic în tratamentul cancerului. Aceasta a presupus elaborarea unor metode de inserare a unor gene în genomul celulelor canceroase cu ajutorul a diverse sisteme de transport sau folosirea altor tehnici care permit anularea expresiei genelor implicate în cancerogeneză sau utilizarea de acid nucleic (fie ADN sau ARN) pentru a influenţa sinteza proteinelor. În teorie este posibilă transformarea celulelor fie somatice sau germinale prin terapia genică, iar terapia genică poate fi întreprinsă atât in vitro cât şi in vivo. Lumea medicală manifestă un interes deosebit pentru introducerea în terapie a unor agenţi antitumorali specifici (anticorpi, inhibitori ai receptorilor factorilor de creştere, antagonişti ai factorilor de transducţie a semnalelor, vaccinurile cu celule care prezintă antigene) în asociere sau nu cu tratamentul chimioterapic.*

Esophageal cancer has a very somber prognosis due to the late diagnosis, the early extension and the fragile nature of the patients, and represents the fourth cause of mortality through cancer, after lung, colon and prostate cancer. Esophageal cancer represents approximately 1% of all the cancers and 6% of all the gastrointestinal cancers. Romania has a low incidence of esophageal cancer in comparison with other countries.(1) Nevertheless, more recent data (1) estimate for Romania 744 new cases per year (at the incidence rate of 3.1 per hundred thousand inhabitants, for both sexes combined) and 681 deaths due to esophageal cancer per year (at the mortality rate of 2.8 for 100,000 inhabitants).

Its frequency has remained constant during the last decade (despite the decrease of the number of epidermoid cancers, an increase of the number of cases of adenocarcinomas has been recorded) as well as despite the progresses of the surgical and oncological treatments, mortality has remained extremely high, the patients being in general elderly, debilitated and malnourished, often cachectic at the time of the diagnosis.

More than 90% of the esophageal cancers are adenocarcinomas or epidermoid cancers, the rest being rare tumours: melanomas, carcinoid tumours, sarcomas, lymphomas. The peculiar lymphatic drainage of the esophagus and the absence of the serous membrane explain the fast and distance formation of metastases.(2)

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SURGICAL TREATMENT

The goal of the oncological surgical intervention for this disease is the resection of the primary tumor and of the drainage lymph nodes representing the golden standard for the treatment of this disease.

Esophagectomy

Esophagectomy represents the main therapeutic attitude, being an aggressive intervention with significant risks. Surgery is the first choice for patients with a resectable tumor, without any signs of locoregional adenopathies, metastases or contraindications (cardiorespiratory defects (3) and malnutrition (4,5)

The choice of the surgical technique depends on several factors:

- the anatomical localization of the lesion, the cervical localization entailing technical difficulties and frequently palliative interventions, whereas those localized in the inferior 1/2 and 1/3 of the esophagus benefitting more frequently from curative interventions; the lesions with a cervical localization are extremely aggressive forms of cancer due to the rich cervical lymphatic network;(6)
- the locoregional and distance extension of the lesion, with the staging of the lesions;
- the surgical procedure used to restore transit, frequently using the stomach, more rarely the colon, both procedures having arguments for and against.

Taking into account the tendency of esophageal submucous lesions, this fact usually requires a subtotal esophagectomy, using a transthoracic or transhiatal approach.

The correct transthoracic approach may be performed through an anastomosis in the upper thorax for smaller tumors and GEJ (the Ivor-Lewis technique), or in the throat for greater lesions. The transhiatal approach avoids thoracotomy and always places the anastomosis in the throat. The distal resection of the esophagus and the reconstruction can also be used, through the left part of the chest or using the thoracoabdominal approach.(7)

Esophagectomy is a radical resection where a mass of tissue with 10cm margins proximally and distally from the tumor includes the thoracic esophagus, the thoracic duct, the azygos vein, the posterior pericardium and the soft tissue in the posterior mediastinum.

Every approach has advantages and disadvantages. The transthoracic technique enables the direct visualization of the tumor and the dissection of more nodal and paraesophageal tissue. The disadvantage of this operation is the cardiopulmonary aggression imposed by thoracotomy and the increased morbidity due to anastomotic fistulae in the thorax. The transhiatal approach has the potential to minimize the respiratory compromise and the anastomotic complications can be more easily monitored and treated. Its disadvantages are the incapacity of performing a complete dissection of the esophagus and of completely visualizing the tumor. The proximity of the trachea over the carina makes it impossible to perform the complete resection of the area for T3 and the total resection of the superior mediastinal ganglia (paratracheal, recurring) is difficult through the thoracic approach.(8)

The radical dissection of the lymph nodes (extensive limphodissection of the superior mediastinal ganglia) requires considerable expertise and the decrease of postoperative complications has not been proved.

The stomach is used frequently for the reconstruction of the esophagus. It can easily be mobilized, it has an excellent vascular intake, and, in almost all the cases it reaches the throat and the base of the tongue. The neoesophagus can be positioned transthoracically or retrosternally. If the stomach cannot be used due to a prior gastrectomy or to a tumor, the colon can be used for the reconstruction. The left colon is frequently used with the vascularization based on the ascending branch of the left colic artery. With the three necessary anastomoses for the restoration of the gastrointestinal continuity, the morbidity is higher, but the functional results are excellent.(9)

The patients presenting with metastases or with tumors in advanced stages are treated palliatively. The palliative treatment should aim, first of all, to improve dysphagia and esophageal obstruction. This can be achieved by several means, including palliative radiations, endoscopic dilation, endoscoping stenting by inserting, under general anesthesia or local oropharyngeal anesthesia, a prosthesis made of plastic, composite or a self expanding metallic stent guided by a metallic thread under radiologic control, endoscopic laser therapy or any another light therapy (e.g. fotodynamic therapy). The self expanding metallic stents are the most efficient as they maintain their position in time and as the size of the tumor increases, one of their qualities residing in the fact that they maintain the permeability of the lumen. Prostheses cannot be used in the case of tumors with a pharyngoesophageal localization, or in the case of tumors localized at the level of the thoracic esophagus which determine multiangular stenoses, or in the case of esocardiac tumors. In these cases, palliative chemotherapy has only a limited role and a marginal impact on survival.

Gene Therapy involves a wide range of types of treatment which use of genetic material for the treatment of cancer.

At present, its application in clinical treatment is an accepted form, representing a global interest project for the study of cancer. However, it is currently an experimental medical treatment.

This method has entailed the development of a technique to insert into the genome of cancer cells genes introduced through different transport systems or through other techniques, which allow the cancellation of the functions of the genes involved in carcinogenesis or the use of nucleic material (nucleic acid: RNA or DNA) to influence protein synthesis. Theoretically it is possible to transform somatic or germinal cells using gene therapy, both *in vivo* and *in vitro.*

In clinical practice, this method is used to inhibit the function of certain genes, to increase immunosuppression, to transfer certain tumor suppressor genes or to inactivate the oncogenes by using antisense mechanisms and to restore mutated genes. In practice, the therapy with suppressor genes or oncogenes is used.

Tumor Suppressor Genes

Tumor suppressor genes are inactivated through genetic or epigenetic modifications of the type of punctiform mutations, deletions (LOH), or promoter methylation. The role of tumor suppressor genes (such as Rb, p53, APC) in the development of neoplasms is essential. This fact has been proved by the reintroduction of one or more types of such genes into the cells, where their function was compromised and where, following this procedure, the neoplastic process was resumed. It was proved experimentally that by restoring the function of the suppressor genes the reversal of the malignant phenotype can be reversed. From this point of view, it is not surprising that many tumors frequently present the loss of heterozygosity (LOH) in chromosomal areas specific for tumor suppressor genes. The loss of heterozygosity (LOH) causes the inactivation of the majority of the tumor suppressor genes which were found in critical chromosomal areas. (1p, 3p, 4, 5q, 9, 11, 13q, 17q si 18q).(10,11)

The loss of the function of certain genes having a tumor suppression role seems to be responsible for the

disappearance of the apoptotic signal in tumor cells, the result being a malignant proliferation. Mutations of the p53 suppressor gene were found in many types of cancers in humans, and, consequently, the reinoculation of this p53 gene in the cancer cells is an effective treatment method. There are several genes which may selectively inhibit the metastatic potential of cancer cells such as connexin, the fibronectin receptor, E-cadherin or nm-23 genes.

Oncogenes

The most frequently activated oncogenes in esophageal cancer are cyclin D1, c-erbB1 and 2, c-myc, c-ras, Int-2/hst-1 and EGFR. The mechanisms for the activation of these oncogenes include: punctiform mutations, amplification, rearrangement and overexpression, out of which amplification and overexpression are the most common.(12)

The ideal of oncogene-targeted gene therapy is the correction of the balance between the proliferative signals by inhibiting the function of some genes involved in maintaining unrestricted proliferation and the acquisition of the metastatic phenotype.

By using antisense mechanisms one can obtain the inactivation of activated dominant oncogenes. These antisense mechanisms are: the use of antisense mRNA molecules, including some oligonucleotides, which prevent transcription by binding to the bicatenar DNA, resulting in the formation of a triple helix, antisense oligonucleotides which prevent the transcription by binding to the sense allele. During this period the current investigation targets are: the inhibition of the RAS oncogene, of the catalytic subunit of TERT, PTTG1. The release of antisense mRNA within a cancer cell has proved to be more efficient than the use of antisense oligonucleotides.

The methods used to inhibit the expression of the oncogenes are:

- The expression of synthetized molecules or of antimolecule antibodies which inhibit the function of the oncogenes intracellularly
- The transfer of antisense nucleotides using short gene sequences or integral DNA
- RNA transfer

In practice the selective activation of prodrugs is used. Once introduced in tumor cells, they block certain genes which are sensitive to these drugs. These genes are also called “suicide” genes.

The therapy with „suicide genes” (prodrug) or the therapy using genes encoding enzymes

„Suicide genes” are the genes usually encoding an enzyme which converts a non-toxic prodrug into a toxic molecule which will lead to the death of the cells in which they are expressed. Among these, the most studied genes were the thymidine kinase gene of the Herpes simplex virus (HSV-TK) associated with Ganciclovrir (GCV) and the cytosine deaminase gene (CD) of Escherichia coli associated with 5- fluorocytosine (5- FC).

The Expression of some pro-apoptotic or cytotoxic genes

The selective transfer of some genes that should result in the destruction of the cancer cells can be achieved using specific mechanisms and without relying on exogenous drugs.

Apoptosis is achieved by using specific ligands or promoters by means of the transfer of the target genes or of their expression in tumor cells. Results can be obtained through the cancellation of the expression of the oncogene genes by means of the ribosymes or the use of immunogenic therapy obtained through the transfer of genes for cytokines resulting from the vaccination with tumor cells or the use of costimulatory molecules.(13,14)

Problems with gene therapy

It is essential that the gene manipulation of the somatic cells not be transmitted to the germinal cells as well, thus blocking its transmission to the descendants.

Problems which have so far remained unsolved in gene therapy:

- Problems related to the use of viral vectors - viruses determine a variety of potential problems for the patient through toxicity, the activation of the inadequate immune and inflammatory response, through the control of the genes and of the problems related to the target- in other words, viruses can induce the disease.
- Multigenic disorders - if cancer appears due to the mutations of a simple gene, then the best treatment is gene therapy. However, most of the times cancers are the result of the mutations of multiple genes, which makes the use of gene therapy difficult.
- The short duration of the action of gene therapy - the fast use of the therapeutic gene introduced into the target cell as well as its functioning during a short period of time and its expression becoming unstable. At present this would be one of the causes why patients have to undergo several successive rounds of gene therapy.
- carcinogenesis - a gene which is integrated into an inadequate place in the genome, for example a suppressor gene, can alter its function and can acquire a mutagenic potential leading to the appearance of tumors.
- The immune response- an activation of the immune system can be triggered, which will determine a decrease in the effectiveness of gene therapy, even an amplification of the response to the viral vectors can be determined, leading to the ineffectiveness of gene therapy.(13,14,15,16)

Gendicine (ro.: gendicina = virusul adeno recombinant) has officially been approved by the Chinese authorities to be used for the squamous-cell carcinoma of the head and neck, and it has also been used as an experiment to establish a clinical trial to treat cancer of the digestive tract (esophagus, stomach, colon, liver, pancreas, gall bladder, rectum), lung cancer, sarcoma, thyroid gland cancer, breast cancer, cervical cancer and ovarian cancer. In addition, patients with advanced cancer, without any other possibility of treatment, are allowed, on a case by case basis, to receive the new drug. Used alongside chemotherapy and radiation, it is argued that Gendicine added years to the lives of the patients and simultaneously improved their quality of life. Gene therapy is a type of treatment which aims to manipulate specific genes in the cells of a patient. In some types of cancer, for example, a particular tumor suppressor gene called p53 is often considered to be defective. During gene therapy, manufactured forms of the normal gene are reintroduced into the patient's tumor cells in order to correct the deficiency and to restore to normal the cell function. In approximately half of all human tumors, this crucial tumor suppressor gene is modified (mutated) and cannot carry on its normal activity of preventing cancer growth.

- Gendicine is made up of two components:
- • p53 genes which function normally
- • An adenovirus carrier or "vector" carrying this p53 gene to the cancer cells.
- Gendicine itself combines a gene called p53, which suppresses tumor formation with a modified common virus. When the product is injected into a tumor, the virus carries the gene to the cancer cells. The gene then asks the tumor cells to commit suicide.
- ***Advexin***. The similarity between Gendicine and Advexin is that both target the p53 gene. The p53 gene is

assumed to induce cell death when the cells begin to over-multiply. In many types of cancer, p53 stops, thus resulting tumors.

- The company manufacturing Advexin has recently announced positive results from phase II. They noted that on a group of patients with head and neck cancer included in the study, some responded to the administration of Advexin with a reduction of the tumor size to more than half, which contributed to an increase of the average survival rate from 6 months without treatment to 41 months after their treatment. Their conclusions were that in the patients where the response to the treatment on tumor size reduction was smaller, however, the average survival rate was improved.

- Recent studies are investigating gene P73. *GRAMD4 mimics p53 and mediates the apoptotic function of p73 at mitochondria p73*, a member of the p53 family, shares high sequence homology with p53 and shows many p53-like properties: it binds to p53-DNA target sites, transactivates p53-responsive genes and induces cell cycle arrest and apoptosis. Apart from this transcription-dependent effect, less is known about the downstream mechanisms through which p73 controls cell fate at the mitochondria. P73-induced apoptosis is mediated by expression of GRAMD4 and translocated to mitochondria. This protein physically interacts with Bcl-2, promotes Bax mitochondrial relocalization and oligomerization, and is highly efficient in inducing mitochondrial membrane permeabilization with release of cytochrome **c** and Smac. Overexpression of p73***α*** and p73***β*** isoforms, but not p53, leads to direct ***GRAMD4*** promoter transactivation. GRAMD4 induces changes in Bcl-2 and Bax protein levels. ***GRAMD4*** transcription is activated in response to cisplatin (cDDP) in a manner dependent on endogenous p73. Using solid tumor xenografts, the ectopic expression of GRAMD4 together with cDDP resulted in enhanced cancer killing. P73 is able to trigger apoptosis via the mitochondrial pathway through a new mechanism using pro-apoptotic GRAMD4 as a mediator, and strongly supporting its p53-like function.

Conclusions:

1. Esophageal surgery involves significant technical and tactical difficulties, in comparison with the surgery of other organs, due to the difficulty of accessing the position of the esophagus and to its relations with vital organs, as well as due to the necessity to mobilize the abdominal viscera for its reconstruction, the optimum solution being esophagectomy with or without lymphadenectomy, associated with chemoradiotherapy.
2. The choice of the surgical technique depends on many factors, such as the anatomical localization of the lesion, the cervical localization entailing technical difficulties and frequently palliative interventions, whereas those localized in the inferior 1/2 and 1/3 of the esophagus benefitting more frequently from curative interventions, the locoregional and distance extension of the lesion, the surgical procedure used to restore transit, frequently the stomach, more rarely the colon, both procedures having arguments for and against and, finally, the contraindications (cardiorespiratory problems and malnutrition).
3. Preoperative chemoradiotherapy may determine an increase in resectability, without changing operative mortality and morbidity, the rate of distance recurrences being considerably reduced through neo-adjuvant chemoradiotherapy, local recurrences remaining unchanged.
4. In order to discover the fundamental mechanism of esophageal carcinogenesis, the degree of expression of many genes must be known, including the genes involved in apoptosis, the genes involved in cellular proliferation, the genes involved in the degradation and repair of DNA and the genes involved in the transmission of the signal in pharyngeal neoplasms, so that the early diagnosis and treatment of esophageal cancer may be achieved, leading to the decrease of the mortality rate.
5. Gene therapy represents a project of worldwide interest for the study of esophageal cancer on which the future treatment of cancer associated with the classical one will be based. At present, the results of gene therapy are promissing and have lead to an increase in the survival and quality of life of the patients who have used such a treatment obtained from genetic engineering. In the future, valuable molecular markers or genes correlated with esophageal cancer will be discovered.

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