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Expression of p21 and bcl-2 proteins in paraffin-embedded preparations of non-small cell lung cancer in stage IIIA after etoposide and cisplatin induced chemotherapy

Lung cancer has now become the leading cause of cancer deaths in both men and women all over the world. Apoptosis, also known as programmed cell death, is a complex, tightly genetically regulated process whereby individual cells commit suicide in a manner that does not injure neighbouring cells or cause any inflammatory reaction. This process directly regulates tumourigenesis (1). Many genes and proteins are involved in this process. The first gene shown to be specifically involved in this process of physiological cell death was *bcl-2* (6). *bcl-2* is an oncogene with antiapoptotic function (7). Although expression of Bcl-2 protein has been reported for a variety of human epithelial malignant tumours, including the lung, the precise biological role of Bcl-2 in the development of malignant tumours is still controversial. In NSCLC (non-small cell lung cancer) most reports found that Bcl-2 expression was associated with favourable clinicopathological characteristics and prognosis, although the antiapoptotic action of Bcl-2 is expected to confer a survival advantage to the cancer cell (5). High levels of *bcl-2* gene expression are found in a wide variety of human cancers and correlate with relative resistance to current chemotherapeutic drugs and radiation (8). Protooncogen *bcl-2* blocks cell death following a variety of stimuli such as UV radiation, chemotherapy agents, dexamethasone and others. Bcl-2 has been reported to cause resistance to anticancer therapy. The next protein involved in apoptosis is $p21^{WAF1/CIP1}$ protein, which suppresses the activity of most complexes of cyclin-dependent kinases with cyclins. The growth suppressor *WAF1* gene, also known as *CIP1* codifies a 21 kd protein, p21, which has been described as the critical downstream effector in the p53-specific pathway of growth control in mammalian cells: p53 overexpression in response to DNA damaging agents promotes the activation of p21, thus inducing growth arrest through inhibition of cyclin-dependent kinases. Expression of p21 is regulated by wild-type *p53* gene (3,4). Recently, it has been shown that *WAF1* can also be induced by p53-independent pathways (2,9). In our study we evaluated an expression of proteins p21 and Bcl-2 in non-small cell lung cancer cells. We examined cancer tissues resected from 30 patients who underwent neoadiuvant chemotherapy. As a control we evaluated the expression of these proteins in tissues from patients treated without chemotherapy.

MATERIAL AND METHODS

We used tissues from non-small cell lung cancers from two groups of patients. The first group was represented by 30 patients who underwent neoadiuvant chemotherapy: Vepesid (intravenously 200mg/m^2/24h) and Cisplatin (intravenously 50mg/m^2/24h) given through three days every 21 days, repeated three times. The surgery was carried out four weeks after chemotherapy was completed. The next was a group of patients treated without chemotherapy, with a surgical method only. Tissue sections were fixed on glass in formalin and embeded in paraffin blocks. We performed four-six micron thick tissue sections with microtome. Then we deparaffinised sections with different concentrations of alcohol (100%, 90%, 80%) and xylen and performed antigen unmasking using standard procedures (by heating at 95°C in 10mM sodium citrate buffer pH 6.0). To asses the expression of p21 and bcl-2 proteins we used immu-

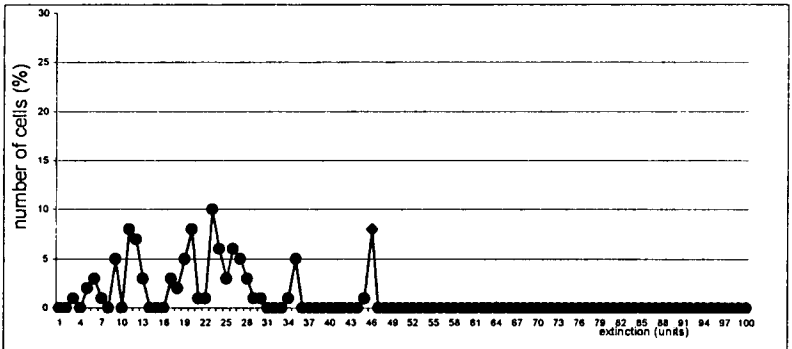

Fig. 1. Extinction of bcl-2 in patients who underwent preoperative chemotherapy

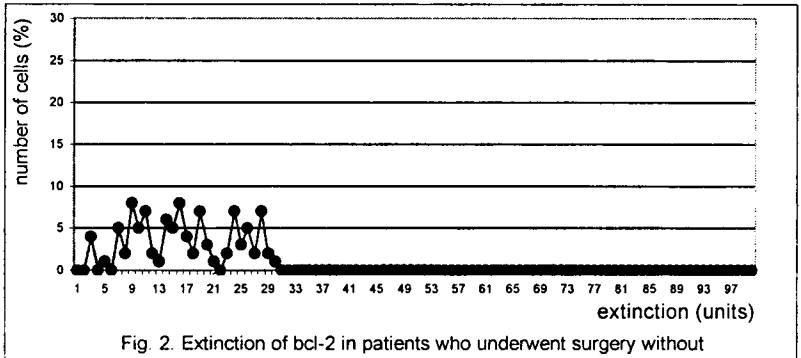

Fig. 2. Extinction of bcl-2 in patients who underwent surgery without preoperative chemotherapy

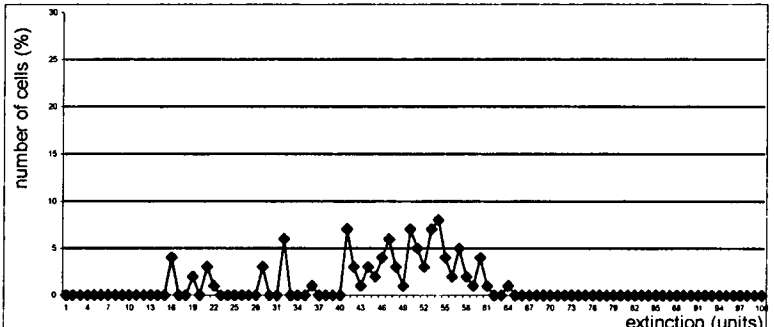

Fig. 3. Extinction of p21 in patients who underwent preoperative chemotherapy

nostaining method with mouse ABC Staining System (Santa Cruz Biotechnology) and primary antibodies bcl-2 and p21 (Santa Cruz Biotechnology). Each procedure was carried out according to the manufacturer's recommendations.

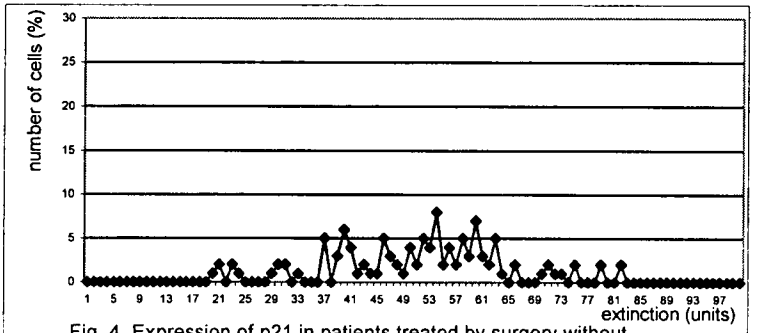

Fig. 4. Expression of p21 in patients treated by surgery without preoperative chemiotherapy

RESULTS AND DISCUSSION

The results were documented by cytophotometry and shown in charts. We analyzed our sections under light microscopy at the and of this procedure. We estimated extinction from 100 cells from each section. Of the 30 cases with NSCLC in the first group, 6 (20%) showed positive cytoplasmatic staining for Bcl-2. The other cases were Bcl-2 negative (80%). In the other group of patients with NSCLC we observed a positive Bcl-2 reaction in 5 cases (15%) and the others were Bcl-2 negative (85%). Expression of p21 protein was modest in the first group of patients and more significant in the other group. In our study we could observe that expression of p21 protein that *in vitro* inhibits growth, clonogenecity and tumorigenecity increased significantly in non-small cell lung cancer cells after preoperative chemotherapy. Expression of bcl-2

was at the same level in both groups of patients and did not change in patients who underwent neoadiuvant chemotherapy.

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SUMMARY

Apoptosis plays an important role in cancer pathogenesis. Several oncogenes and antioncogenes regulate this process. Loss of their normal function leading to cell resistance to apoptosis seems to be a key factor of neoplasm development. In tumour cells, programmed cell death is a spontaneous process and its intensity increases after chemio-, radio- and hormonotherapy. Amongst several genes and their products, *bcl-2* and *p21* genes play a significant role in the process. *p21* gene product, cyclin-dependent kinase inhibitor, along with *p53* gene take part in cell cycle regulation. Our study aimed at evaluating p21 and Bcl-2 protein expression in the cells of patients afflicted with stage IIIA of non-small cell lung cancer who underwent neoadiuvant chemotherapy (three courses of Vepesid and Cisplatin). Protein expression was evaluated in slides of tissue material obtained before pharmacological treatment (during bronchofiberoscopy) and after three courses of Vepesid and Cisplatin (during surgical tumour resection). Protein activity in tissue slides was conducted using histochemical method with labelled antibodies (immunoperoxidase staining procedure). The control material was obtained from patients who had not undergone inductive chemotherapy. The results were documented as photographs and presented as charts after extinction level measurement using cytophotometric technique. Decrease in Bcl-2 protein activity and increase in p21 protein level in tumour cells of patients after inductive chemotherapy were observed.

Ekspresja białek p21 i bcl-2 w komórkach raka niedrobnokomórkowego płuc w stadium IIIA po chemioterapii indukcyjnej

Apoptoza jest procesem odgrywającym bardzo duże znaczenie w patogenezie raka. W jej kontrolę zaangażowanych jest kilka onkogenów i antyonkogenów. Utrata prawidłowej funkcji tych genów, prowadząca do oporności komórek na apoptozę, wydaje się ważnym elementem rozwoju nowotworów. Programowana śmierć komórek następuje spontanicznie w komórkach nowotworowych, a jej nasilenie zwiększa się pod wpływem leczenia chemioterapią, radioterapią i hormonoterapią. Spośród wielu genów i ich produktów białkowych w procesie tym odgrywają istotną rolę geny *bcl-2* i *p21*. Produkt białkowy genu *p21*, będący inhibitorem cyklinozależnych kinaz, uczestniczy wraz z genem *p53* w regulacji cyklu komórkowego. W celu oceny znaczenia białka p21 i Bcl-2 badaliśmy ekspresję tych białek w komórkach niedrobnokomórkowego raka płuc w stadium IIIA pacjentów poddanych neoadjuwantowej chemioterapii (Vepesid z Cisplatyną w trzech kursach). Ocenę ekspresji tych białek przeprowadzaliśmy w preparatach tkankowych wykonanych z materiału pobranego od pacjentów przed leczeniem farmakologicznym (materiał pobierano podczas bronchofiberoskopii) oraz po trzech kursach Vepesidu z Cisplatyną (materiał pobrany podczas resekcji chirurgicznej guza). Następnie stosując metodę histochemiczną z wykorzystaniem znakowanych przeciwciał (ang. *immunoperoxidase staining procedure*) ocenialiśmy aktywność badanych białek w preparatach tkankowych. Kontrolę stanowił materiał pobrany podczas zabiegu operacyjnego od pacjentów, którzy nie zostali poddani chemioterapii indukcyjnej. Wykonaliśmy dokumentację fotograficzną z przeprowadzonych badań, a wyniki przedstawiliśmy w formie wykresów po zmierzeniu poziomu ekstynkcji za pomocą cytofotometru. Obserwowaliśmy spadek aktywności białka Bcl-2 oraz wzrost poziomu białka p21 w komórkach nowotworowych, pobranych od pacjentów poddanych uprzedniej chemioterapii indukcyjnej.