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Expression of p53 gene in non-small cell lung cancer in stage IIIA in patients after neoadiuvant chemotherapy (Vepesid and Cisplatyna)

The p53 tumour suppressor protein is involved in multiple central cellular processes, including transcription, DNA repair, genomic stability, senescence, cell cycle control and apoptosis. The p53 protein is clearly a component of one of the pathways activated in response to DNA damage. The molecular pathway between DNA damage and p53 accumulation is not understood (6). The physiological function of wild-type p53 is to "guard the genome" by transcriptional activation of genes leading to cell cycle arrest (1). Current research defines the biochemical pathways through which p53 induces cell cycle arrest and apoptosis. Knowledge of these fundamental processes leads to the identification of molecular targets toward which multimodality cancer therapies, using chemotherapeutic, immunotherapeutic and gene-therapeutic strategies can be based (4). p53 is a nuclear phosphoprotein capable of binding to specific DNA sequences and activating specific target genes. It was shown that wild type p53 confers drug sensitivity *in vitro*, whereas p53 loss is associated with resistance (7). Gene *p53* directly affects an expression of downstream genes that regulate sensitivity to apoptosis (3).

MATERIAL AND METHODS

We used the tissue from cancers of two groups of patients with non-small cell lung cancer (NSCLC) at the stage IIIA. The first group was represented by 30 patients who underwent neoadiuvant chemotherapy (Vepesid and Cisplatin). Thirty patients treated surgically, without chemotherapy represented the other group. Chemiotherapeutic drugs were given intravenously at doses 50mg/m^2/24h Cisplatin and 200mg/m^2/24h Vepesid. Patients received drugs through three days every 21 days three times. Surgery was performed four weeks after the last cycle of chemotherapy.

Tissue sections were fixed on glass in formalin and embedded in paraffin blocks according to standard procedures. 4–6 micron slides were made using microtome. Next we deparaffinised slides using a number of concentrations of alcohols and xylen. Using proteinase K at the concentration 1ug/ml (30 minutes) we prepared sections for *in situ* hybridisation. Antigen unmasking was performed by heating at 95°C in 10nM sodium citrate buffer pH 6.0.

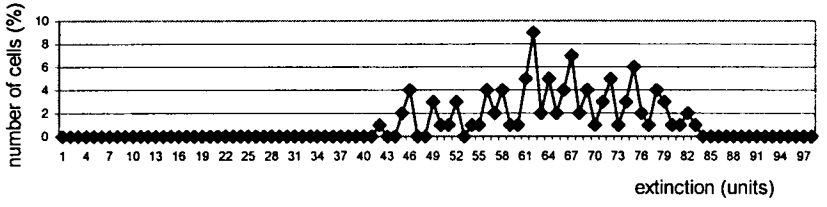

Fig. 1. Extinction of non- small lung cancer cells in patients who underwent chemotherapy. TUNEL reaction

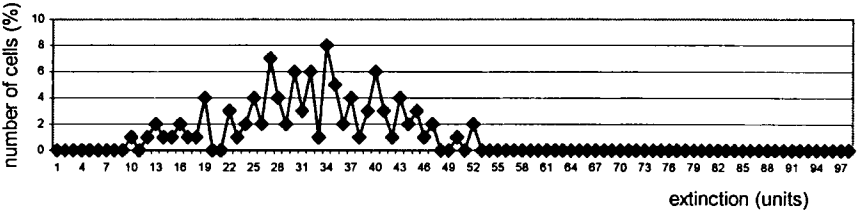

Fig. 2. Extinction of non- small lung cancer cells without chemotherapy. TUNEL reaction

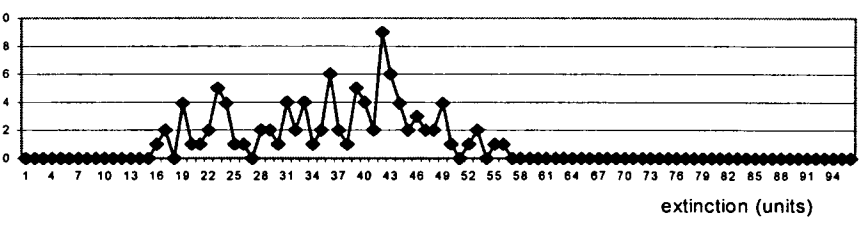

Fig. 3. Extinction of non- small lung cancer cells in patients who underwent chemotherapy. In situ hybrydisation. Expression of p53

TUNEL (Tdt- mediated dUTP nick end labeling), also called *in situ* Labeling System (ISEL). It is enzymatic *in situ* labelling of apoptosis induced DNA strand breaks. Terminal deoxynucleotidyl transferase (TdT) was used for the incorporation of labelled nucleotides to DNA strand breaks *in situ* (2, 5). To detect apoptosis we used *in situ* Cell Death Detection Kit, AP (Roche Molecular Biochemicals). Each stage of this procedure was carried out according to the manufacturer's recommendations. We analyzed our slides under fluorescence microscopy

(OLYMPUS) to detect DNA fragmentation after In Situ End Labeling reaction and under light microscopy after staining procedure. Then we measured extinction of cells by cytophotometry.

In situ hybridisation. To asses expression of *p53* mRNA we used Oligo Colour Kit (Amersham Pharmacia Biotech) and *p53* antisens oligonucleotide labeled with 12 dUTP. Each stage of this procedure was carried out according to manufacturer's recommendations.

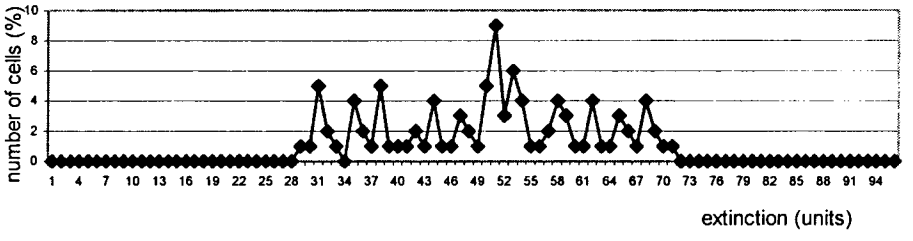

Fig. 4. Extinction of non- small lung cancer cells without chemotherapy. In situ hybrydisation. Expression of p53

RESULTS AND DISCUSSION

The results were documented by photography and cytophotometry and shown in charts. We documented both fluorescein and staining reaction by photography and measured extinction of about 100 cells of each section and presented results at the charts. We observed positive TUNEL reaction in the first group of patients (patients operated on after neoadiuvant chemotherapy) and negative TUNEL reaction in the other group (treated only with surgical methods). Expression of *p53* gene was modest in the first group and more significant in the other group. p53 protein suppresses tumour formation in vivo at least in part through its ability to induce cell death. Loss of p53 appears to play an important role in the resistance to chemotherapy. The relative sensitivity of cancer cells to induction of apoptosis by drugs is modulated by p53 in many cases. Our study confirms the assumption that induction of p53 activity by DNA-damaging agents can decrease the resistance of cells to programmed cell death.

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SUMMARY

Apoptosis (programmed cell death) plays a very important role in the development regulation, homeostasis maintenance as well as in the origin of many diseases, including neoplasms. This process is genetically regulated and reflected in characteristic morphological and biochemical changes taking place in cells. The process is considered to be of great significance in tumour originating and growth as well as in tumour cell response to chemotherapy. There are many genes and their products that are involved in apoptosis. The following genes: *p53, bcl-2* and *p21* seem to have the greatest significance. Our study aimed at evaluating *p53* gene expression in non-small-cell lung cancer patients after neoadiuvant chemotherapy. We examined the tissue material from 35 patients after three-cycle inductive chemotherapy (Vepesid and Cisplatin). The material was obtained before chemotherapy during bronchofiberoscopy and four weeks after drug treatment during surgery. The control group comprised patients who had not undergone inductive chemotherapy. After deparaffinising of tissue slides, gene *p53* activity using *in situ* hybridisation technique was evaluated. Moreover, apoptosis valuation with TUNEL method was performed. The results were documented as photographs. Gene p53 activity level was estimated using cytophotometric technique. Our study revealed significantly higher percentage of cells undergoing apoptosis and increased gene p53 activity in tumour tissue slides of patients after neoadiuvant chemotherapy.

Ekspresja genu *p53* w komórkach raka niedrobnokomórkowego płuc w stadium IIIA po chemioterapii neoadiuwantowej

Apoptoza, nazywana również programowaną śmiercią komórek (ang. *programmed cell death*), odgrywa bardzo ważną rolę w procesie rozwoju, homeostazie, a także w wielu chorobach, między innymi nowotworowych. Jest to proces regulowany genetycznie, którego odzwierciedleniem są charakterystyczne zmiany morfologiczne i biochemiczne, zachodzące w komórkach. Uważa się, iż ma ona bardzo duże znaczenie w powstawaniu i rozwoju nowotworów oraz w odpowiedzi komórek nowotworowych na chemioterapię. Bierze w niej udział szereg genów i ich produktów białkowych, spośród których istotne znaczenie wydają się mieć geny *p53, bcl-2* oraz *p21*. W naszej pracy ocenialiśmy ekspresję genu *p53* w komórkach niedrobnokomórkowego raka płuc u pacjentów poddanych chemioterapii neoadjuwantowej. Badaliśmy materiał tkankowy pochodzący od 35 pacjentów poddanych chemioterapii indukcyjnej, w której skład wchodziły Vepesid i Cisplatyna, stosowane w trzech cyklach. Materiał pobierano od pacjentów przed chemioterapią w trakcie bronchofiberoskopii oraz po upływie czterech tygodni od zakończenia leczenia farmakologicznego podczas zabiegu operacyjnego. Jako kontrolę wykorzystano materiał pobrany od pacjentów, którzy nie zostali poddani uprzedniej chemioterapii indukcyjnej. Po deparafinizacji preparatów tkankowych wykonanych z bloczków parafinowych ocenialiśmy aktywność genu *p53* w badanych komórkach za pomocą metody hybrydyzacji *in situ*. Ponadto przeprowadzaliśmy ocenę apoptozy metodą TUNEL. Wyniki zostały zarejestrowane w postaci dokumentacji fotograficznej. Poziom aktywności genu *p53* ocenialiśmy mierząc poziom ekstynkcji za pomocą cytofotometru. W naszych badaniach obserwowaliśmy znamiennie wyższy odsetek komórek ulegających apoptozie oraz większą aktywność genu *p53* w preparatach tkankowych pochodzących z guzów pacjentów poddanych chemioterapii neoadjuwantowej.