Diagnostic Utility of CD66c in Lung Adenocarcinoma-Associated Malignant Pleural Effusion: Comparison with CEA, CA 19-9, and CYFRA 21-1

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Background: Various tumor markers have been evaluated in malignant pleural effusions, but not CD66c. This study evaluated the ability of CD66c to discriminate lung adenocarcinoma-associated malignant pleural effusions (LA-MPEs) from benign effusions compared with carcinoembryonic antigen (CEA), cancer antigen (CA) 19-9, and CYFRA 21-1. Methods: Pleural effusions were collected from 37 patients with LA-MPE, and 43 with benign conditions. The levels of CD66c, CEA, CA 19-9, and CYFRA 21-1 were assayed by enzyme immunoassay. The protein expressions of CD66c, CEA, CA 19-9 were evaluated by immunocytochemistry. Results: The levels of all tumor markers were significantly higher in LA-MPE than in benign effusions (p < 0.001). CEA had the best diagnostic values, with a sensitivity of 97.7%, specificity of 78.4%, and areas under the curve (AUC) of 0.896. CA 19-9 with a sensitivity of 97.7%, specificity of 56.8%, and AUC of 0.781, CYFRA 21-1 with a sensitivity of 81.4%, specificity of 78.4%, and AUC of 0.839, and CD66c with a sensitivity of 97.6%, specificity of 62.2%, and AUC of 0.795 were measured. CEAs combined with CYFRA 21-1 increased the AUC to 0.925, with a sensitivity of 86.1% and specificity of 89.2%. The immunocytochemistry using cell blocks revealed that CD66c was the most sensitive marker. Conclusions: By immunoassay, CEA combined with CYFRA 21-1 had the best diagnostic performance. CD66c had comparable sensitivity to CEA, but lower specificity and AUC. Immunocytochemistry showed CD66c was more sensitive than CEA or CA 19-9. Enzyme immunoassay and immunocytochemistry support CD66c as a potential tumor marker to differentiate LA-MPE from benign effusions.

Key Words: CEACM6 protein, human; Adenocarcinoma of lung; Pleural effusion

Etosis-like Cell Death in Human Cancer

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Background: Etosis is a peculiar form of cell death distinguished from both apoptosis and necrosis and depends on the generation of reactive oxygen species (ROS) by NADPH oxidase. This ROS-dependent death allows neutrophils to fulfill their antimicrobial function. We would like to introduce an etosis-like cell death in human cancer. Methods: We selected several cases of human cancer including carcinoma and lymphoma. All the cases had the necrotic area on hematoxylin and eosin staining. Fluorescence stain was performed using DAPI, anti-histone, anti-laminin A/C in paraffin-embedded tissue sections as well as staurosporine-treated human cervical cancer cell line, HeLa, which were examined on a laser scanning confocal microscope LSM710. Results: Human cervical cancer cell line, HeLa cells released their DNA and histone and attached danger-associated molecular patterns in a stressful condition, quite similar to dying neutrophils secreting extracellular traps. Moreover, these characteristic cell death was observed in paraffin-embedded tissue sections irrespective of types of cancer. Conclusions: We suggest that etosis is one of the cell death mechanism in human cancer.

Key Words: Neoplasms; Etosis; Necrosis; Apoptosis

Comparison of Stanniocalcin-1 Expression in Coronary Plaques of Acute Myocardial Infarction or Stable Angina

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Background: Stanniocalcin-1 (STC1) is involved in fundamental biological processes such as angiogenesis, inflammation, and wound healing, but little is known about its expression in human coronary atherosclerotic plaques or its relationship to plaque instability. Methods: STC1 expression was examined in the culprit coronary plaques of 70 patients with acute myocardial infarction (AMI; n=49) or stable angina (n=21) who underwent directional coronary atherectomy. The specimens were stained with hematoxylin and eosin, STC1-specific antibodies, and endothelial cell, macrophage, and smooth muscle cell markers. Results: The baseline characteristics of the two groups of patients were largely similar. CD31- and CD68-immunopositive areas, indicative of the presence of endothelial cells and macrophages, respectively, were proportionately larger while areas immunopositive for an actin, as a smooth muscle cell marker, were proportionately smaller in the AMI group than in the stable angina group. The proportion of STC1-immunopositive areas was significantly greater in the AMI group than in the stable angina group (20.0% [8.2-29.0%] vs 8.8% [3.9-19.4%]; p = 0.022). Areas positive for STC1 were independently correlated with those immunopositive for CD31 (r = 0.54, p < 0.001) and CD68 (r = 0.53, p < 0.001). STC1 immunoreactivity colocolated with CD31- and CD68-immunopositive cells. Conclusions: STC1 is differentially expressed in the culprit coronary plaques of patients with AMI versus those with stable angina. STC1 may play a role in plaque instability.

Key Words: Coronary disease; Plaque stability; Teleocalcin

SB365 Isolated from Pulsatilla koreana Induce Apoptosis in Human Brain Glioblastoma Cells

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AP23-PP-0001 Free Paper (Poster)
Background: Development of small molecules that inhibit cancer progression is especially important in brain tumor by potentially being able to cross the blood brain barrier. SB365, *Pulsatilla saponin D* from the root of *Pulsatilla koreana* Pulsatil has known to inhibit proliferation and induce apoptosis in hepatocellular carcinoma and colon cancer cell lines. This study was planned to determine the effect that SB365, has on apoptosis in human glioblastoma cell lines. Methods: We examined the effect of SB365 on human glioblastoma cell lines: U87MG, A172, and T98G. Apoptosis was measured by evaluating terminal deoxynucleotidyl transferase dUTP nick end labeling assay and DAPI nuclear staining. The expression of cleaved poly(ADP-ribose) polymerase (PARP), Bcl-2, Bax, and cleaved caspase-3 were assayed by western blot analysis in cells treated with SB365. Results: High rates of apoptosis were induced by SB365 in the glioblastoma cell lines. The proliferative rates of brain glioblastoma cells were definitely suppressed by SB365 in a dose dependent and time dependent manner. The outcome of Western blotting showed that protein expressions of cleaved PARP, bax and cleaved caspase-3 were increased, however Bcl-2 was markedly decreased. SB365 demonstrated potent anti-angiogenic activity and markedly reduced the expression of hypoxia-inducible factor 1a. Conclusions: In summary, SB365 inhibit of growth and proliferation through induction of apoptosis in human glioblastoma cell lines. These findings indicate that SB365 might contribute to the growth inhibitory activities in vitro and also might be useful as a good pharmaceutical candidate for use in the treatment of brain tumor.

Key Words: SB365; Apoptosis; Glioblastoma

Coexpression of mTOR and Glycogen Synthase Kinase 3β in Normal Human Epithelial Cells in an Organ-Specific Manner

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Background: Mammalian target of rapamycin (mTOR) and glycogen synthase kinase 3β (GSK3β) are downstream targets of phosphatidylinositol 3-kinase (PI3K)/AKT pathway and are crucial in cellular metabolism and proliferation. mTOR and GSK3β are deregulated in many disease conditions and inhibitors of these proteins are used as targeted therapeutic agent. In spite of their importance in various diseases and tumors, the expression of mTOR and GSK3β in normal human organ has been largely unknown. Methods: We examined the immunohistochemical localization of phospho-mTOR (p-mTOR) and phospho-GSK3β (p-GSK3β) in human tissues, and also compared the expression pattern of p-mTOR and p-GSK3β with that of the cytokeratin (CK) 7 and CK20. Results: We found that p-mTOR and p-GSK3β staining was restricted to the epithelial cells of breast and pancreatic duct, distal tubule and collecting duct of kidney, gastrointestinal tract, endometrial gland, fallopian tube, epididymis, rete testis, secretory cell of prostatic gland, umbrella cell of urinary tract and mesothelial cells in adult. In fetus, the expression pattern of p-mTOR and p-GSK3β was similar to that of the adult with addition of expression in the fetal pulmonary. The staining pattern of p-mTOR, p-GSK3β and CK7 was overlapped except for gastrointestinal tract where CK7 was negative. Conclusions: Phosphorylated form of mTOR and GSK3β coexist in normal human ductal or glandular epithelial cells and they may be associated with epithelial structuring although they are in different spots in PI3K/AKT pathway.

Key Words: TOR serine-threonine kinases; Glycogen synthase kinase 3β; Epithelial; Coexpression

Automatic Indexing of Surgical Pathology Report Using Term Frequency-Inverse Document Frequency

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Background: Indexing of surgical pathology report depending on human effort is time-consuming and less representative, and may cause considerable inconsistencies. Terms used in the indexing may be too coarsely granular in describing various features of each sample. Translational researchers increasingly request for human samples suited for their delicate research design. Methods: We empirically compared the features of more representative terms to less representative ones in each of 30 surgical pathology reports. Many of those features were attributable to the frequencies of the terms in the report and in every report. After preprocessing of surgical pathology report, we applied term frequency-inverse document frequency (tf-idf), which is a commonly used metric in indexing of web documents. According to the tf-idf, five most representative terms were produced for each report. We compared those automatically indexed terms and human-indexed terms and evaluated the effectiveness of our methods by concordance rate and appropriateness in 100 randomly selected pathology reports. Results: We can collect representative indexing terms better describing the cases in more detail. Although many of not representative terms appeared, most of them were resolved after preprocessing. The representativeness was improved as more data corpus was collected. The mean concordance rate between human indexing and automatic index was 63.6%. When appropriateness was compared by human experts, automatic indexing was more appropriate in 55.8%. Conclusions: We proved the adequacy of tf-idf in indexing of pathology report, which is simple, semantically clear, and computationally feasible. Translational research will be more promoted with the adoption of these methodologies.

Key Words: Automatic indexing; Pathology report; Term frequency-inverse document frequency

Cost-Free Manual Tissue Microarray Could Be as Perfect as Semi-automated One

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Background: Development of small molecules that inhibit cancer progression is especially important in brain tumor by potentially being able to cross the blood brain barrier. SB365, *Pulsatilla saponin D* from the root of *Pulsatilla koreana* Pulsatil has known to inhibit proliferation and induce apoptosis in hepatocellular carcinoma and colon cancer cell lines. This study was planned to determine the effect that SB365, has on apoptosis in human glioblastoma cell lines. Methods: We examined the effect of SB365 on human glioblastoma cell lines: U87MG, A172, and T98G. Apoptosis was measured by evaluating terminal deoxynucleotidyl transferase dUTP nick end labeling assay and DAPI nuclear staining. The expression of cleaved poly(ADP-ribose) polymerase (PARP), Bcl-2, Bax, and cleaved caspase-3 were assayed by western blot analysis in cells treated with SB365. Results: High rates of apoptosis were induced by SB365 in the glioblastoma cell lines. The proliferative rates of brain glioblastoma cells were definitely suppressed by SB365 in a dose dependent and time dependent manner. The outcome of Western blotting showed that protein expressions of cleaved PARP, bax and cleaved caspase-3 were increased, however Bcl-2 was markedly decreased. SB365 demonstrated potent anti-angiogenic activity and markedly reduced the expression of hypoxia-inducible factor 1a. Conclusions: In summary, SB365 inhibit of growth and proliferation through induction of apoptosis in human glioblastoma cell lines. These findings indicate that SB365 might contribute to the growth inhibitory activities in vitro and also might be useful as a good pharmaceutical candidate for use in the treatment of brain tumor.

Key Words: SB365; Apoptosis; Glioblastoma

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Key Words: TOR serine-threonine kinases; Glycogen synthase kinase 3β; Epithelial; Coexpression

Coexpression of mTOR and Glycogen Synthase Kinase 3β in Normal Human Epithelial Cells in an Organ-Specific Manner

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Background: Mammalian target of rapamycin (mTOR) and glycogen synthase kinase 3β (GSK3β) are downstream targets of phosphatidylinositol 3-kinase (PI3K)/AKT pathway and are crucial in cellular metabolism and proliferation. mTOR and GSK3β are deregulated in many disease conditions and inhibitors of these proteins are used as targeted therapeutic agent. In spite of their importance in various diseases and tumors, the expression of mTOR and GSK3β in normal human organ has been largely unknown. Methods: We examined the immunohistochemical localization of phospho-mTOR (p-mTOR) and phospho-GSK3β (p-GSK3β) in human tissues, and also compared the expression pattern of p-mTOR and p-GSK3β with that of the cytokeratin (CK) 7 and CK20. Results: We found that p-mTOR and p-GSK3β staining was restricted to the epithelial cells of breast and pancreatic duct, distal tubule and collecting duct of kidney, gastrointestinal tract, endometrial gland, fallopian tube, epididymis, rete testis, secretory cell of prostatic gland, umbrella cell of urinary tract and mesothelial cell in adult. In fetus, the expression pattern of p-mTOR and p-GSK3β was similar to that of the adult with addition of expression of these two proteins in the fetal pulmonary. The staining pattern of p-mTOR, p-GSK3β and CK7 was overlapped except for gastrointestinal tract where CK7 was negative. Conclusions: Phosphorylated form of mTOR and GSK3β coexist in normal human ductal or glandular epithelial cells and they may be associated with epithelial structuring although they are in different spots in PI3K/AKT pathway.

Key Words: TOR serine-threonine kinases; Glycogen synthase kinase 3β; Epithelial; Coexpression
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**Background:** Manual tissue microarray (TMA) construction has been introduced to avoid the high cost of automated and semi-automated techniques. Although many researchers tried to develop simple techniques for TMA construction, these methods were complicated, difficult to apply, and expensive. We tried to introduce a new simple inexpensive method for manual TMA construction with high quality reaching that of expensive ones. **Methods:** Two conventional mechanical pencil tips of different diameters (0.7 and 0.9 mm) were used. The smaller for preparing holes of the recipient block, and the larger for puncture of the desired cores from donor blocks. A source of mild heat was used during construction to ease puncture. Blocks were incubated at 38°C overnight, in order to increase stability and quality of the blocks. **Results:** Three high density TMA blocks with 206 cores/block were constructed from different tissues with high stability. We successfully performed hematoxylin and eosin slides and immunostaining without substantial tissue cylinder loss. **Conclusions:** Our no-cost mechanical pencil tip technique raises the quality of manual TMA blocks, increase the number of cores per block and improve the stability of the cores within the paraffin block. This new modified technique is a good alternative of expensive machines in many laboratories.

**Key Words:** Manual; Tissue array analysis; Colorectal neoplasms

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Mongolian-Korean Pathologists Cooperation Results and Perspectivity

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**Background:** Mongolia is the central Asian developing country with total 2.8 million population and there are working only less than one hundred pathologists. Mongolia has one state medical university named Health Sciences University of Mongolia and it has 6 years of undergraduate medical program and 1.6 year pathology residence course including forensic medicine after graduation of university. Up to now there are no specialized post graduate training for pathology after residence course. In order to constantly refresh knowledge of our pathologists we established the Mongolian Society of Cytopathology and Histopathology in 2007. **Methods:** Our joint workshop aim is to strengthen Mongolian and Korean relationship and expand Mongolian pathologist’s knowledge and experience, introduction of new technology, quality assurance, upgrade the skill of pathological diagnoses, training of Mongolian doctors in Korean medical centers. **Results:** The Mongolian Society of Cytopathology and Histopathology have organized 5 joint seminars and workshops together with the Korean Society of Pathologists and the Korean Society for Cytopathology since September 2007 with different targeted topics mainly intended to educate and expand experience of our pathologists. **Conclusions:** Mongolian doctors are very thankful on the reached results of joint cooperation and wish to continue and expand our cooperation in the future.

**Key Words:** Mongolia; Korea; Joint workshop; Pathology; Cytology

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Polymerase Chain Reaction-Based Analysis of Herpes Simplex and Varicella zoster Viral Coinfections in Skin Biopsy Specimens

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**Background:** Simultaneous coinfections of herpes simplex virus (HSV) and varicella zoster virus (VZV) have been considered as a rare event, about 1% in western area. There have been only a few case collective studies on the incidence and clinical manifestation of simultaneous coinfection. The aim of present study is to investigate the prevalence and clinical characteristics of cutaneous HSV and VZV coinfections. **Methods:** PCR analyses for HSV type1 and HSV type2, and nested-PCR for VZV were carried out separately on skin biopsy specimens from 106 patients at Seoul St. Mary’s Hospital from January 2008 to March 2013. Cases showing positive results for two or three subtypes of virus in same biopsy specimen were defined as co-infection, which results were analyzed with clinical characteristics. **Results:** See tables. Table 1. Comparison of single and coinfected patients. **Conclusions:** Unexpectedly high percentage of dual infection may result from regional difference or specimens which used for examination or method of detection. Coinfections by HSV and VZV may be more prevalent than clinical expectations, therefore the recognition of their high prevalence is necessary for pathologists as well as clinicians.

**Key Words:** Polymerase chain reaction; Herpesvirus 3, human; Herpes Simplexvirus; Coinfection

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Mesenchymal Stromal Cells Promote Infiltrative Tumor Growth in Tumor Xenograft Model

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Tumor microenvironment has been well recognized to have important role for tumor proliferation and metastasis. However, little is known about potential influence of species-specific microenvironment for human and mice model. Here, we investigated the impact of mesenchymal stromal cell (MSC) as a potential factor that can influence tumor microenvironment. First, identical CFC-equivalent number of mouse (WEHI164) and rat sarcoma cell (RR1022) were injected subcutane-
miR-550 Inhibits Cell Proliferation and Progression of Breast Cancer by Targeting MAPK1 and MAPK3

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Background: Breast cancer is the most common cancer and the principle cancer-leading cause of women worldwide. MicroRNAs (miRs) are critical regulators in breast carcinogenesis and progression. MiR-550a has been proved to play an important role in some cancers but remains unknown in breast cancer. This study is designed to analyze regulatory effects of miR-550a in breast cancer. Methods: After miR microarray-based and real-time polymerase chain reaction comparison, miR-550a-3p was revealed as the most declined miR in breast cancer tissues and cells than normal controls. The biological effects of miR-550a-3p were investigated in cell line model. Bioinformatic prediction showed that miR-550a-3p binding sites on their 3’ untranslated region. Clinical significance was evaluated with in situ hybridization and immunohistochemistry. Results: There is a declined trend of miR-550a-3p among normal breast tissues, primary, invasive and metastatic breast cancers. Similar pattern also found in breast cancer cell lines. And it directly targeted and silenced two well-documented oncogenes of breast cancer, MAPK1 and MAPK3. Ectopic miR-550a-3p inhibited MAPK1 and MAPK3 expression, downstream ERK signaling, and therefore reduced breast cancer cell proliferation, survival, migration, and invasion. Conversely, anti-miR-550a-3p conferred inverse effects. Within case-control study, lower miR-550a-3p, higher MAPK1 or MAPK3 were associated with higher incidence risk and poorer survival. Conclusions: MiR-550a-3p plays a tumor-suppressor role in breast cancer initiation and progression and may function as a useful diagnostic or prognostic marker of breast cancer.

Key Words: Breast neoplasms; MicroRNAs; Mitogen-activated protein kinase 1; Mitogen-activated protein kinase 3

Secreted miR-19A Communication Enhances Migratory and Invasive Ability of Urothelial Cell Carcinoma Cell Lines

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Background: Secreted miRNAs confer biological effects via cell-cell communication, such as the miR17/20 cluster has shown to govern cellular migration and invasion and therefore increase tumor metastatic ability. This study is purposed to evaluate whether secreted miRNAs of high-grade cancer cells induce aggressive transformation of low-grade cancer cells. Methods: Since urothelial cell carcinoma (UCC) of bladder occurs in a storage organ that offers an accessible circumstance for secreted miRNAs; accordingly, we carried out a cell-to-cell miRNA transferring design in UCC cell lines. The high-grade T24 cell was used as secreted miRNA donor and its conditioned medium was used to induce aggressive transformation of low-grade RT4 cell. RT4 cultured with T24 conditioned medium were continued for six months, and cell morphology or miRNAs/target genes variations compared each month. Results: As T24 cells released the miR-17-92 cluster, especially miR-19a, into the extracellular environment, RT4 cells received secreted miR-19a and transformed into irreversibly higher aggressive morphologies and behaviors, that is, more spindle sharp and higher migratory/invasive abilities. The expression level of PTEN and CYKD, two miR-19a targets and potent tumor invasion inhibitors, were significantly reduced in RT4 cells by secreted miR-19a, which was indicating that secreted miRNA via cell-cell communication can govern cellular migration and invasion in RT4 cells. Conclusions: In this study, we found the cell-to-cell miRNA transferring enhanced cell migration and invasion of UCC cells, and miR-19a may function as important regulators within the aggressive transformation.

Key Words: Urothelial cell carcinoma; Secreted miR; MIRN19 microRNA

Expression of Matrix Metalloproteinase 2 and Fascin in Highly Invasive Glioblastoma Cells

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Key Words: Urothelial cell carcinoma; Secreted miR; MIRN19 microRNA, human
**Background:** Glioblastoma is lethal because of local invasion into surrounding normal brain along blood vessels and myelinated fiber tracts of white matter that contain extracellular matrix proteins. To identify mechanisms related with invasion of glioblastoma, we separated more invasive glioblastoma cells from less invasive ones. **Methods:** We isolated more invasive subpopulation (U87-Inv) and comparatively less invasive one (U87-Non) from the U87MG cell line through merosin (laminin-2) coated transwell filters. We compared the results of wound healing assay, gelatin zymography for matrix metalloproteinases (MMPs), proliferation assay, expression of integrin receptors (reverse transcription polymerase chain reaction), and expression of fascin and actin (western blot and immunofluorescence) between the two subpopulations. **Results:** U87-Inv demonstrated faster wound healing, which was associated with increased expression of MMP2. The comparative phenotypic analysis showed spindle shaped appearances for U87-Inv cells and more extension shape after merosin coating with decreased phosphorylation of ERK1/2 and decreased proliferation index compared to U87-Non cells. Moreover, fascin, the actin-bundling protein, was expressed at a higher level in U87-Inv cells. U87-Inv cells showed increased expression of α1, and some also of α7, and β1 integrin subunits. **Conclusions:** More invasive glioblastoma cells showed marked lamellipodial protrusions expressing both fascin and actin, which was associated with MMP2 activation, lower proliferative activity, and increased expression of α1, α7 and β1 integrin subunits.

**Key Words:** Glioblastoma; Laminin; Matrix metalloproteinase 2; Fascin; Pseudopodia

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**Comparative Study of Microenvironmental Effect of Asbestos Fibers on Pulmonary Fibroblast: A Real-Time Cell Analysis**

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**Background:** Several mineral fibers are both fibrogenic and carcinogenic to lungs. Among the mineral fibers, asbestos is well known for its cytotoxicity. In asbestosis lungs, asbestos bodies are frequently recognized in the pulmonary interstitium, a medium of lung fibrosis and microenvironment for carcinogenesis. Although cytotoxic effects on pulmonary alveolar cells, the first target of inhaled fibers, have been studied, the etiology of pathological effects on the microenvironment of lung fibrosis is yet to be elucidated. **Methods:** Fibers from different origins were classified by examining the experimental X-ray diffraction patterns against the known database files. A real-time cell analyzer (xCELLigence, Roche diagnostics) was utilized to observe the cellular reactions to lung fibroblasts (IMR-90), which was considered the microenvironment of lung fibrosis. Fibers of serpentine (chrysotile) and amphibole (amosite and crocidolite) minerals were studied in concentrations of 10, 50, and 100 µg/mL. The cellular reaction was observed by confocal laser microscope. **Results:** The investigated fibers showed different cytotoxic effects on IMR-90. Chrysotile showed the cytostatic effect in all concentrations, crocidolite caused steady cell death, and amosite had an effect similar to the mitosis interference. **Conclusions:** In conclusion, all types of asbestos fibers showed cytotoxic effects on the lung fibroblasts in vitro, however, in different manners. Further investigations are necessary to fully understand the underlying mechanisms of the cellular interactions and their ramifications in vivo.

**Key Words:** Asbestos; Fibroblast; Cytotoxicity; Lung; Tumor microenvironment