Dr. Bellyei Szabolcs **68469. sz. OTKA pályázat**

1. Magyar összefoglaló

Munkacsoportunk folyamatosan végzi a szövet-, vizelet- és szérumminták szisztémás gyűjtését különféle daganatokban, ahogy azt a pályázat munkatervében felvázoltuk. Együttműködést építettünk ki az Idegsebészeti Klinikával, a PTE Nőgyógyászati Klinikával és a Sebészeti Klinikával. A mintavételek az Intézetek által előre egyeztetett protokollok alapján történnek és egy tűzvonalat építettünk ki, amely mindig biztosítja a megfelelő személyeket, hogy bármikor gyorsan megszervezhető legyen a minták pontos levétele, eljutása a műtőből a Biokémia Intézet hűtőjéig.

Korábbi közleményeinkben már beszámoltunk egy új kis molekulasúlyú fehérje azonosításáról Hsp16.2. Vizsgáltuk a fehérje expresszióját különféle humán neuroectodermalis tumorokban. Végül azt találtuk, hogy a Hsp16.2 megjelenése a különféle neuroectodermális agytumorokban korrelál a szövettani grádussal. Ezáltal, mint lehetséges tumor marker érdemes a fehérje további vizsgálata. Eredmények közölve. A fehérje további pathológiás szöveti vizsgálatai folyamatban vannak egyéb tumorokban (rectum-, nyelőcső- tumorok).

Munkacsoportunk írta le a „tail-interacting protein of 47 kDa TIP47" fehérjét, feltételezett funkcióját. Előzetes publikációink alapján tovább vizsgáltuk a fehérje esetleges klinikai alkalmazását nőgyógyászati dysplasiakban, primer tumorokban és metasztázisok esetén. Vizsgálataink alapján a TIP47 szérumszintjének monitorozása alkalmas lehet a cervix daganat nyomon követésére, esetleges recidívák észlelésére. Az eredmények publikáció formájában megjelentek.

A TIP47 fehérje funkcionális vizsgálatait tovább folytatjuk a molekuláris biológia módszereivel. A fehérje cDNS-ét NIH3T3 fibroblaszt sejtekbe klónoztuk és ezáltal vizsgáljuk a sejtekre kifejtette hatást, nézzük a jelátviteli rendszerben történő változásokat (AKT, GSK, P38, ERK, Bax, Bcl-2) apoptózis indukció és oxidatív stressz hatására. Korábban leírtuk, hogy a HeLa sejtek nagy mennyiségben termelik a TIP47 fehérjét, így ezen sejteket TIP47 dsiRNA kezelve, vizsgáljuk a fehérje elnyomása következtében történő változásokat. A fehérjének a védelmi oldalon van szerepe, antiapoptótikus hatása van. Az eredmények 2 külön publikáció formában megjelentek

Tovább folytatjuk az általunk publikált „heme-binding protein 2/ SOUL" fehérje funkcionális vizsgálatát. A fehérje számítógépes szekvencia analízise alapján BH3 domént találtunk a szekvenciában, így feltételezzük, hogy szintén szerepe lehet a sejthalál mechanizmusában. Eredmények alapján a fehérjének szerepe lehet a „mitochondrial permeability transition" – folyamataiban, elsősorban a nekrotikus útvonalak aktiválásában involvált. A publikáció megjelent.

További célkitűzésünk volt nyelőcső-daganatos betegek szövettani mintájában tumor asszociált fehérjék keresése, amelyek expresszió ja utalhat a kemo-radioterápiára adott válaszra. Eredményeink alapján a kemo-radioterápiára jobban reagáló daganatoknál alacsonyabb Hsp16.2, Hsp90, és pAKT szintet kaptunk, míg magasabb bax/bcl-2 arányt és fokozott SOUL expressziós szintet találtunk. Következtetéseink, hogy lokálisan előrehaladott nyelőcsőtumoros betegeknél a neoadjuváns kezelések eredményességének előrejelzésére tumor asszociált fehérjék biopsziás mintából történő vizsgálata ígéretes módszernek tűnik. A publikáció megjelent.

Továbbá vizsgáltuk, hogy rectumtumoros betegeknél a neoadjuváns, szimultán radiokemoterápiával elérhető terápiás válasz (downstaging) és kezelés előtti biopsziás minták

immunhisztológiai vizsgálattal történő különféle fehérjék expressziós szintjei között, találunk-e összefüggést. Egyelőre 100 beteg anyaga van feldolgozás alatt. A kezelés előtti biopsziás mintákat vizsgáljuk immunhisztokémiával. A fent már említett tumormarker jelölteket tanulmányozzuk. A publikáció elküldve, várjuk a választ.

Számos közleményben számoltunk már be a PP13/galectin 13 fehérjéről, mely fehérje további vizsgálatai eredményeit közleményben foglaltuk össze, a publikáció elfogadva.

Összességében az OTKA pályázat kiindulásakor kitűzött feladatokat messzemenőkig teljesíteni tudtuk. Amit mi sem bizonyít jobban, hogy az elmúlt 4 évbe az eredményeinket magas impakt faktoros lapokban tudtuk közölni. A 4 év alatt számos hazai és külföldi konferencián beszámoltunk az eredményeinkről. Az eltel 4 év alatt 3 db. Ph.D hallgató, akik fel vannak tüntetve, mint OTKA részvevők már megvédték a téziseiket, elnyerték a Ph.D fokozatot. További 1 hallgató az ősz folyamán fogja megvédeni a Ph.D disszertációját.

2. Angol nyelvü szakmai összefoglaló

All of the results of the working-group was published in high value (with high impact factor) international journals in English language.

Small heat shock proteins are molecular chaperones that protect proteins against stress-induced aggregation. They have also been found to have anti-apoptotic activity and to play a part in the development of tumors. Recently, we identified a new small heat shock protein, Hsp16.2 which displayed increased expression in neuroectodermal tumors. Our aim was to investigate the expression of Hsp16.2 in different types of brain tumors and to correlate its expression with the histological grade of the tumor. Immunohistochemistry with a polyclonal antibody to Hsp16.2 was carried out on formalin-fixed, paraffin-wax-embedded sections using the streptavidin-biotin method. 91 samples were examined and their histological grade was defined. According to the intensity of Hsp16.2 immunoreactivity, low (+), moderate (++), high (+++) or none (-) scores were given. Immunoblotting was carried out on 30 samples of brain tumors using SDS-polyacrylamide gel electrophoresis and Western-blotting. Low grade (grades 1-2) brain tumors displayed low cytoplasmic Hsp16.2 immunoreactivity, grade 3 tumors showed moderate cytoplasmic staining, while high grade (grade 4) tumors exhibited intensive cytoplasmic Hsp16.2 staining. Immunoblotting supported the above mentioned results. Normal brain tissue acted as a negative control for the experiment, since the cytoplasm did not stain for Hsp16.2. There was a positive correlation between the level of Hsp16.2 expression and the level of anaplasia in different malignant tissue samples. In conclusion Hsp16.2 expression was directly correlated with the histological grade of brain tumors, therefore Hsp16.2 may have relevance as becoming a possible tumor marker.

Pozsgai E, Gomori E, Szigeti A, Boronkai A, Gallyas F Jr, Sumegi B, Bellyei S
Correlation between the progressive cytoplasmic expression of a novel small heat shock protein (Hsp16.2) and malignancy in brain tumors.
BMC CANCER 7: 233 *(2007)*
IF: 2.709

The aim of this next study was to find a possible clinical use of the tail-interacting protein of 47 kDa (TIP47) and further document its expression in smear cytology, different cervical dysplasias, invasive cervical cancer and metastasis. A new polyclonal anti-TIP47 antibody was developed and used on smears and histological cervix sections of sixty women with different cytological pathologies. Serum TIP47 level of patients with cervical intraepithelial neoplasia (CIN) or carcinoma in stage IIb, IIIa, and IIIb was monitored during treatment.TIP47 was expressed weakly in the dysplasias, stronger in invasive tumors and in lymph node metastasis. In patients with cervical carcinoma, the serum TIP47 level was found to be elevated; it decreased after therapy and elevated again in relapse. According to our results, TIP47 could be a good clinical marker for the early detection in blood of the recurrence of cervical carcinoma.

*Szigeti A, Minik O, Hocsak E, Pozsgai E, Boronkai A, Farkas R, Balint A, Bodis J, Sumegi B, Bellyei S Preliminary Study of TIP47 as a Possible New Biomarker of Cervical Dysplasia and Invasive Carcinoma ANTICANCER RES **29**: (2)*717-724 *(2009)*
IF: 1.390

Galectin-13 transcripts have been identified in several normal and malignant tissues, but the physiological function of galectin-13 is still poorly understood. Here, we presented the evidence for its possible role in promoting cell death in the U-937 human macrophage cell line. Transfection of U-937 human macrophages by a galectin-13 cDNA-containing mammalian expression vector increased the galectin-13 level and sensitized the cells to stress stimuli. Galectin-13 overexpression facilitated paclitaxel-induced cell death and nuclear translocation of apoptosis-inducing factor (AIF) and endonuclease-G without inducing mitochondrial cytochrome-c release or caspase-3 activation. Immunoblot and immunofluorescence data showed that overexpression of galectin-13 induced long-term activation of c-Jun N-terminal kinase (JNK) and p38-mitogen-activated protein kinase (MAPK) pathways, as well as activation of apoptosis signal-regulating kinase-1 (Ask-1) kinase while it suppressed paclitaxel-induced long-term activation of the phosphatidilylositol-3-kinase (PI-3K)-Akt and extracellular signal-regulated kinase (ERK1/2) cytoprotective pathways. In addition, pharmacological inhibition of JNK and p38-MAPK pathways protected the cells from paclitaxel-induced cell death. All this data indicate that galectin-13 overexpression promoted apoptosis presumably by activating the Ask-1 kinase-JNK and p38-MAPK pro-apoptotic pathways and by suppressing the PI-3K-Akt and ERK1/2 cytoprotective pathways.

Boronkai A, Bellyei S, Szigeti A, Pozsgai E, Bognar Z, Sumegi B, Gallyas F Jr
Potentiation of paclitaxel-induced apoptosis by galectin-13 overexpression via activation of Ask-1-p38-MAP kinase and JNK/SAPK pathways and suppression of Akt and ERK1/2 activation in U-937 human macrophage cells.
*EUR J CELL BIOL **88**: (12)*753-763 *(2009)*
*IF: 3.955**

We identified a sequence homologous to the Bcl-2 homology 3 (BH3) domain of Bcl-2 proteins in SOUL. Tissues expressed the protein to different extents. It was predominantly located in the cytoplasm, although a fraction of SOUL was associated with the mitochondria

that increased upon oxidative stress. Recombinant SOUL protein facilitated mitochondrial permeability transition and collapse of mitochondrial membrane potential (MMP) and facilitated the release of proapoptotic mitochondrial intermembrane proteins (PMIP) at low calcium and phosphate concentrations in a cyclosporine A-dependent manner in vitro in isolated mitochondria. Suppression of endogenous SOUL by diced small interfering RNA in HeLa cells increased their viability in oxidative stress. Overexpression of SOUL in NIH3T3 cells promoted hydrogen peroxide-induced cell death and stimulated the release of PMIP but did not enhance caspase-3 activation. Despite the release of PMIP, SOUL facilitated predominantly necrotic cell death, as revealed by annexin V and propidium iodide staining. This necrotic death could be the result of SOUL-facilitated collapse of MMP demonstrated by JC-1 fluorescence. Deletion of the putative BH3 domain sequence prevented all of these effects of SOUL. Suppression of cyclophilin D prevented these effects too, indicating that SOUL facilitated mitochondrial permeability transition in vivo. Overexpression of Bcl-2 and Bcl-x(L), which can counteract the mitochondria-permeabilizing effect of BH3 domain proteins, also prevented SOUL-facilitated collapse of MMP and cell death. These data indicate that SOUL can be a novel member of the BH3 domain-only proteins that cannot induce cell death alone but can facilitate both outer and inner mitochondrial membrane permeabilization and predominantly necrotic cell death in oxidative stress.

Szigeti A, Hocsak E, Rapolti E, Racz B, Boronkai A, Pozsgai E, Debreceni B, Bognar Z, Bellyei S, Sumegi B, Gallyas F Jr
Facilitation of mitochondrial outer and inner membrane permeabilization and cell death in oxidative stress by a novel Bcl-2 homology 3 domain protein.
J BIOL CHEM ***285****: (3)*2140-2151 *(2010)*
*IF: 5.520***

Tail-interacting protein (TIP47, also named PP17) has been implicated in lipid droplet metabolism and in the development of late endosomes, to date however, no data about its possible role in regulating cell death processes has been available. Here, we provide evidence for the role of TIP47 in the regulation of mitochondrial membrane stability and cell death. Overexpression of TIP47 protected NIH3T3 cells from taxol-induced cell death, while suppression of TIP47 by siRNA facilitated cell death. TIP47, but not its truncated form, t-TIP47, decreased taxol-induced cell death as determined by propidium iodide and fluorescent Annexin V staining. Recombinant TIP47, but not t-TIP47, partially prevented taxol-induced depolarization of mitochondria in vitro. Overexpression of TIP47, but not its truncated form, prevented the taxol-induced nuclear and cytoplasmic translocation of AIF and Endonuclease G, as well as the taxol-induced depolarization of mitochondria in NIH3T3 cells. Furthermore, overexpression of TIP47 facilitated Bcl-2 expression and suppressed Bax expression in taxol-treated cells. These data show that besides its previously known functions, TIP47 is involved in the regulation of mitochondria-related cell death by directly stabilizing the mitochondrial membrane system and by favorably affecting the expression of Bcl-2 homologues. Since TIP47 is overexpressed in certain tumors, it is possible that TIP47 contributes to the development of cytostatic resistance.

Hocsak E, Racz B, Szabo A, Pozsgai E, Szigeti A, Szigeti E, Gallyas F Jr, Sumegi B, Javor S, Bellyei S. TIP47 confers resistance to taxol-induced cell death by preventing the nuclear translocation of AIF and Endonuclease G. Eur J Cell Biol. 2010 Aug 11. [Epub ahead of print] PubMed PMID: 20708296.
IF: 3.314

We found that overexpression of tail interacting protein of 47kDa (TIP47), but not its truncated form (t-TIP47) protected NIH3T3 cells from hydrogen-peroxide-induced cell death, prevented the hydrogen-peroxide-induced mitochondrial depolarization determined by 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethyl-benzimidazolylcarbocyanine iodide (JC1), while suppression of TIP47 in HeLa cells facilitated oxidative-stress-induced cell death. TIP47 was located to the cytoplasm of untreated cells, but some was associated to mitochondria in oxidative stress. Recombinant TIP47, but not t-TIP47 increased the mitochondrial membrane potential (Deltapsi), and partially prevented Ca(2+) induced depolarization. It is assumed that TIP47 can bind to mitochondria in oxidative stress, and inhibit mitochondria mediated cell death by protecting mitochondrial membrane integrity.

Hocsak E, Racz B, Szabo A, Mester L, Rapolti E, Pozsgai E, Javor S, Bellyei S, Gallyas F Jr, Sumegi B, Szigeti A. TIP47 protects mitochondrial membrane integrity and inhibits oxidative-stress-induced cell death. FEBS Lett. 2010 Jul 2;584(13):2953-60. PubMed PMID: 20556887.
*IF: 3.541***

Possible predictive markers of response to neoadjuvant radiochemotherapy (NRCT) of esophageal cancer have been identified. Patient biopsies were obtained from both tumor and normal tissue before the NRCT of locally advanced esophageal squamous cell carcinoma. Protein solutions were separated and immunoblot analysis was performed with heat shock protein (Hsp)16.2, heme-binding protein 2 (SOUL), BCL2-associated X protein (Bax), B-cell-associated leukemia protein 2 (Bcl-2) and heat shock protein 90 (Hsp90) antibodies. Following NRCT, the patients were restaged according to the Response Evaluation Criteria In Solid Tumors (RECIST). Following resections the pathological down-staging was evaluated. Clinical restaging revealed a response rate of 65%. Pathological examination revealed down-staging in 30% and 25% of the cases for the T and N categories respectively. Compared to the normal esophageal mucosa, a decreased expression of Hsp16.2, Hsp90 and SOUL proteins and an increased Bax/Bcl-2 ratio was found in the responding tumors. Hsp16,2, Hsp90 and SOUL expression and Bax/ Bcl-2 ratio correlates to the efficacy of NRCT and predict outcome in patients with locally advanced squamous-cell esophageal cancer.

Farkas R, Pozsgai E, Bellyei S, Cseke L, Szigeti A, Vereczkei A, Marton S, Mangel L, Horvath OP, Papp A. Correlation between Tumor-associated Proteins and Response to Neoadjuvant Treatment in Patients with Advanced Squamous-cell Esophageal Cancer. Anticancer Res. 2011 May;31(5):1769-75. PubMed PMID: 21617238 IF: 1.390

This final work is under publication. The status: SUBMITTED. The response to neoadjuvant chemoradiotherapy (CRT) varies greatly in patients suffering from locally advanced rectal cancer. It was our aim to correlate the response to CRT with the pretreatment expression of Heat shock protein 90 (Hsp90), small Heat shock protein 16.2 (sHsp 16.2), phospho-Akt (p-Akt), Growth hormone-releasing hormone receptor (GHRH-R) and Heme-binding protein 2 (SOUL) in order to try to identify one or more as a predictive marker. 69

patients receiving combined CRT for locally advanced rectal cancer were examined retrospectively. Surgical resection was carried out 6-9 weeks following CRT. The histopathological response to neoadjuvant treatment was determined according to the modified Mandard score. Using immunohistochemistry we investigated the relationship between the expression of the five cited proteins in the preoperative samples as well as various clinical parameters and the histopathologic regression of the tumors. 31 patients (48%) were good responders and 33 patients (52%) were found to respond poorly to neoadjuvant therapy. Among patients undergoing surgery 7 weeks following CRT there were significantly more good responders than among patients who underwent surgery sooner (63% vs 37%). High levels of expression of GHRH-R and Hsp90 were shown to be significantly correlated with minor or absent histological regression. GHRH-R and Hsp90 were found to be independent predictive factors of histopathological response to neoadjuvant RCT. Since GHRH-R antagonists and Hsp90 inhibitors are currently being tested as potential anticancer agents, our study implies the possible elaboration of an effective and individualized treatment for poor responders.