Results: Western blots demonstrated decreased levels of ZFP161 protein in Zfp161 knockout and shRNA knockdown cells. Simultaneously, decreased c-MYC protein levels were observed in Zfp161 knockout and shRNA knockdown cells compared with wild type and knockdown control cells. Using qPCR, decreased c-Myc RNA levels were observed in Zfp161 knockout cells compared to wildtype cells and decreased c-Myc RNA levels were observed in shRNA knockdown cells compared to control cells. Our results show that knockout and knowdown of Zfp161 results in a significant decrease in c-Myc expression in human cells. The decrease of c-Myc in Zfp161 knockout and knockdown cells translated into a robust decrease of *in vitro* cell survival in colony formation assays.

Conclusions: Zfp161 is a regulator of the c-Myc oncogene in human cells. Zfp161 knockout and knowdown cells show a robust decrease of cell survival *in vitro*.

Legal entity responsible for the study: Zhenkun Lou.

Funding: Mayo Clinic.

Disclosure: All authors have declared no conflicts of interest.

https://doi.org/10.1016/j.annonc.2020.08.176

25P The role of Speedy/RINGO in between MAPK and AKT pathways in SH-SY5Y neuroblastoma cells

O. Tanriverdi[1], Y. Kaya[2], S. Kucukvardar[3], A. Yildiz[1]

[1]Department of Medical Oncology, Mugla Sitki Kocman University Faculty of Medicine, Mugla, Turkey; [2]Department of Molecular Biology and Genetics, Mugla Sitki Kocman University Faculty of Science, Mugla, Turkey; [3]Faculty of Science and Letters, Istanbul Technical University, Istanbul, Turkey

Background: Triggering and overactivation of extracellular signal-regulated kinases/mitogen-activated protein kinase(MAPK) and phosphatidylinositol 3-kinase/protein kinase B(AKT) signaling pathways are one of the most important factors in the formation of many types of cancer, including neuroblastoma, by reducing the effectiveness of treatment and contributing to development of resistance to chemotherapy during the treatment process. There is evidence reinforcing the possibility that these two signaling pathways are communicating and interacting with each other in carcinogenic process. Yet it is unknown what is there at the heart of this interaction. There are studies giving insights about certain cell cycle regulators such as Speedy/RINGO which may be involved in the intersection of these signaling pathways. In these studies, connection of Speedy/RINGO with AKT and MAPK pathways is studied partially and also these studies are performed with cell and tissue types other than neuroblastoma. Thus, to date there is not any data available showing the connective role of Speedy/RINGO in between AKT and MAPK pathways and the aim of this study is to show the connective role of Speedy/RINGO between AKT and MAPK pathways in neuroblastoma cells.

Methods: In this study, MAPK pathway was firstly inhibited with U0126 chemical and expression of Speedy/RINGO was analyzed. A corresponding decrease in the expression of Speedy/RINGO and phosphorylation of AKT were shown. This data was confirmed with siRNA silencing of Speedy/RINGO.

Results: Showed that Speedy/RINGO inhibition caused a significant decrease in CyclinA and CDK2 expression levels and AKT phosphorylation amounts. It has also shown that these inhibition treatments significantly reduce the viability of cancer cells.

Conclusions: All data obtained from this study provide crucial information for the first time about the connecting and overactivating function of Speedy/RINGO in between AKT and MAPK pathways, thereby giving insights to choose appropriate molecular targets for diagnosis and treatment of many cancer types.

Legal entity responsible for the study: The authors.

Funding: This work was supported by the Scientific Research Project Office of Mugla Sitki Kocman University (Project Numbers 17/251 and 17/023).

Disclosure: All authors have declared no conflicts of interest.

https://doi.org/10.1016/j.annonc.2020.08.177

26P Coexpression of E- and N-cadherins as a sign of epithelial-mesenchymal transition (EMT) in prostate cancer (PCa)

M. Puchinskaya[1], D. Prokopenia[2]

[1]Out-Patient Department, Minsk City Clinical Oncologic Dispensary, Minsk, Belarus; [2]General Ecology, Biology, and Environmental Genetics, International Sakharov Environmental Institute of Belarusian State University, Minsk, Belarus

Background: EMT is a process important for malignant tumor metastasis and drug resistance. Various molecules have been studied as markers of EMT, but still none of them can readily define cells more capable of aggressive behaviour. Expression of various markers differs in cancer cells. Several experimental works have shown that simultaneous presence of both epithelial and mesenchymal markers is important for metastasis. We aimed to assess coexpression of E- and N-cadherins (cad) in PCa cells as a sign of EMT.

Methods: A training cohort of PCa samples was stained using double immunofluorescence (dIF) technique. 4 μm thick slides of tissues obtained during radical prostatectomy were used for staining. Primary antibodies to E-cad (BioGenex, 1:150 dilution) and N-cad (ThermoFischerScientific, 1:1500) and secondary antibodies (ThermoFischerScientific, 1:200) conjugated with Alexa Fluor 488 and 555 probes were applied. Slides were studied using Nikon 5500 microscope. Patterns of markers coexpression were assessed.

Results: Staining pattern of both cadherins was predominantly membranous with entire membranes being stained in cancer cells, in some cases cytoplasmic staining was also seen. E-cad staining was homogenous and present in both non-cancerous glands (NCG) and PCa (down-regulated to different extent in the latter). Opposingly, N-cad staining was very patchy, the marker being present in only few cells, also both in PCa and NCG, in some cases staining in the latter was even more prominent. Either entire gland or only some cells were stained. Staining intensity was strong to moderate. As there were no cells with E-cad absence, cadherins co-expression was seen in all N-cad-positive cells and the percentage of coexpressing cells was, correspondingly, the same as N-cad+ cells. No significant signs of stronger E-cad down-regulation in N-cad positive cells compared to adjacent N-cad-negative was noted. Coexpression of cadherins was seen in $<5\%$ of PCa cells in 75% of cases.

Conclusions: Coexpression of epithelial and mesenchymal cadherins in PCa is dependent on N-cad expression that was present in a limited number of cells. It can be speculated that these rare cells are the main source of further PCa progression and metastasis.

Legal entity responsible for the study: M. Puchinskaya.

Funding: Belarusian Republican Foundation for Fundamental Research.

Disclosure: All authors have declared no conflicts of interest.

https://doi.org/10.1016/j.annonc.2020.08.178

27P Laryngopharyngeal reflux affects tumour immune microenvironment in carcinoma of larynx

Y. Kizim[1], D. Zabolotnyi[2], V. Kizim[1], D. Zabolotna[2], O. Sulaieva[3]

[1]Department of Head and Neck Cancer, Kolomiychenko Institute of Otolaryngology of National Academy of Medical Sciences of Ukraine, Kyiv, Ukraine; [2]Department of Otolaryngology, Kolomiychenko Institute of Otolaryngology of National Academy of Medical Sciences of Ukraine, Kyiv, Ukraine; [3]Department of Histopathology, Laboratory of Pathology "CSD Health Care", Kyiv, Ukraine

Background: One of the risk factors of laryngeal cancer (LC), besides human papillomavirus (HPV) and smoking, is laryngopharyngeal reflux (LPR), which rate has been progressively rising worldwide. LPR is associated with low pH that can affect tumor immune microenvironment (TIME) in patients with LC. However, little is known about the effect of LPR on the TIME in LC. The goal of the study was to assess the impact of LPR on TIME of tumor infiltrating T-lymphocytes and macrophages in LC.

Methods: A total of 63 males with HPV-negative pT1-2 squamous cell LC were enrolled in the study. According to the results of 24-h pH monitoring, patients were subdivided into two groups: without (the 1st group, 30 patients) and with coexisting LPR (the 2nd group, 33 patients). TIME was assessed immunohistochemically by counting the number of T-lymphocytes (CD3), T-cytotoxic cells (CD8) and T-regulatory cells (Treg; FOXP3), M1 (CD68) and M2 macrophages (CD163) in the tumor and in the intact mucosa (IM) of the larynx taken from the tumor-negative margins.

Results: LC with coexisting LPR demonstrated higher inflammatory infiltration of tumors and IM than in patients without LPR. However, no statistically significant differences were found between the number of CD3+ and CD8+ cells within LC of the 1st and 2nd groups though density of CD3 cells in IM was higher in the 2nd group ($P=0.003$). In contrast, LPR was associated with increased amount of immunosuppressive Treg cells ($P=0.008$). In addition, numerous CD68+ macrophages were found within LC of both groups. However, M2 macrophages density was much higher in LC ($P<0.001$) and IM ($P=0.021$) of the 2nd group patients. A negative correlation between pH values obtained during pH monitoring and number of tumor-associated CD163-positive macrophages ($r=0.71$; $P<0.001$) supports the association between LPR-related milieu acidity and M2-macrophages polarization in LC.

Conclusions: LPR causes chronic inflammation in the laryngeal mucosa and alters the TIME in LC, facilitating M2-macropheges polarization and increasing Treg cells number that can impact tumor biological behavior and immune escape mechanisms.

Legal entity responsible for the study: Kolomiychenko Institute of Otolaryngology of NAMS of Ukraine.

Funding: Has not received any funding.

Disclosure: All authors have declared no conflicts of interest.

https://doi.org/10.1016/j.annonc.2020.08.179