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Abstract #1

Session	Basic & Preclinical Science Session
Title	The impact of protein arginine methyltransferase 5 mutations on carcinogenesis
Authors	Mihály Székely; Attila Makai; Ilka Keller; Dániel Horváth; Evelin Major; Ádám Ungvári; Klaudia Tóth; Beáta Lontay
Affiliation (University / Department)	University of Debrecen, Faculty of Medicine, Department of Medical Chemistry
Trustee of the paper	Mihály Székely
Introduction	Over- and under-activation of enzymes responsible for maintaining the cell cycle and gene expression is a major cause of tumor development and can be triggered by mutations and abnormal post-translational modifications. The protein arginine methyltransferase 5 (PRMT5) catalyzes the symmetric arginine dimethylation of histone proteins, resulting in the inhibition of gene expression. It is regulated by RhoA-activated kinase (ROK) and myosin phosphatase (MP) via the Thr80 phosphorylation of its MYPT regulatory subunit. Mutations in the Thr80 regulatory side chain of PRMT5 have been identified in several tumor types using the COSMIC database.
Aim of the study	The aim of our studies is to investigate the effect of mutations in PRMT5 Thr80 biochemically.
Material and methods	We transfected tsA201 cells with a plasmid encoding Flag-tagged wild-type and mutant PRMT5 and purified them by Flag affinity chromatography. By mass spectrometry analysis, a new phosphorylation site, PRMT5 Ser74, was described for PRMT5 Thr80 mutant proteins under ROK treatment compared to wild-type PRMT5 enzyme. To investigate the effect of PRMT5 Thr80 mutations on enzyme activity, overexpressed proteins were bound to affinity gels. In vitro ROK kinase assay was conducted on the immobilized PRMT5 and then examined for phosphorylation by Western blotting.
Results	While WT PRMT5 showed no increase in the phosphorylation of Ser amino acid residues upon ROK treatment, an increase was detected in Thr80 mutants. The mutant PRMT5 showed clear PRMT5 Ser phosphorylation by in vitro ROK enzyme activity assay, which was reduced by the addition of MP. We analyzed the amount of histone substrates bound by WT and mutant PRMT5 proteins and found that ROK induced mutant PRMT5 to bind significantly more histone substrates, which was not altered by further phosphorylation. We validated our results with a liver carcinoma tissue array and other tumor cell lines using an anti phospho-Ser74 antibody and observed a grade- and status-dependent correlation in the phosphorylation of tissue samples.
Conslusions	Our data suggest that mutations in tumors may regulate the increased activity of PRMT5 by the amount of substrate bound by the enzyme and that this may result from spatial structural changes and exposure to new activating phosphorylation sites caused by the mutations. Supported by: NTP-HHTDK-22-0051

Abstract #2

Session	Internal Medicine Session
Title	Investigation of thyroid involvement in patients treated with primary Raynaud's phenomenon
Authors	Mihály Székely; Gábor Papp; Zoltán Csiki
Affiliation (University / Department)	University of Debrecen, Faculty of Medicine, Department of Internal Medicine
Trustee of the paper	Mihály Székely
Introduction	Raynaud's phenomenon (RP) is characterized by the vasospasm of the fingers in response to cold or psychological stress, leading to a characteristic discoloration of the skin color of the fingers. It is caused by an imbalance between vasoconstrictor and vasodilator factors. A distinction is made between primary and secondary forms, the latter associated with various underlying diseases, including autoimmune conditions. As the development of Raynaud's phenomenon may be one of the first clinical signs of a systemic autoimmune disease, early detection and detailed investigation of patients is crucial.
Aim of the study	Our retrospective study focused on analyzing laboratory markers measured in patients suspected of having Raynaud's syndrome and then were followed up.
Material and methods	Among the patients examined and monitored for suspected primary Raynaud's phenomenon in our department between 2012 and 2022, 216 female (mean age 43.25 ± 15.71 years) patients with known sTSH and vitamin D levels were included in our study after the exclusion of systemic autoimmune disease.
Results	Among the patients studied, 59.26% had a confirmed thyroid involvement (e.g., hypothyroidism, goiter, carcinoma) or an abnormal sTSH level within a mean of 4.72 ± 1.89 years from the date of the first Raynaud's attack. It should be noted that 31.25% of affected patients did not have a history of thyroid disease diagnosed before the first Raynaud's phenomenon. Plasma or serum vitamin D3 levels were below the reference range in a significant proportion of subjects (83.33%).
Conslusions	In the case of RP, detailed thyroid disease screening is essential, as a large proportion of patients have thyroid involvement at first presentation. In addition, the increased risk of developing thyroid disease should be considered during follow-ups. Furthermore, our results suggest that testing of vitamin D3 levels should always be considered in patients with suspected primary Raynaud's phenomenon, and regular oral supplementation is recommended in case of deficiency. Supported by: NTP-HHTDK-22-0051

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