

## CROATIAN INTERNATIONAL PUBLICATIONS

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**Mise J, Dembitz V, Banfic H, Visnjic D. Combined inhibition of PI3K and mTOR exerts synergistic antiproliferative effect, but diminishes differentiative properties of rapamycin in acute myeloid leukemia cells. *Pathol Oncol Res.* 2011 Sep;17(3):645-56.**

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A novel strategy has been suggested to enhance rapamycin-based cancer therapy through combining mammalian target of rapamycin (mTOR)-inhibitors with an inhibitor of the phosphatidylinositol 3-kinase PI3K/Akt or mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) pathway. However, recent study demonstrated the potentiating effect of rapamycin on all-trans-retinoic acid (ATRA)-mediated differentiation of acute myelogenous leukemia (AML) cells, prompting us to investigate the effects of longitudinal inhibition of PI3K/Akt/mTOR signaling pathway on both proliferation and differentiative capacity of AML. In NB4, HL-60, U937 and K562 cell lines, rapamycin exerted minimal antiproliferative effects, and combining PI3K inhibitor LY 294002 and rapamycin inhibited proliferation more than LY 294002 alone. Rapamycin potentiated differentiation of ATRA-treated NB4 cells, but the combination of rapamycin and LY 294002 inhibited the expression of CD11b in both ATRA- and phorbol myristate acetate (PMA)-stimulated cells more than PI3K inhibitor alone. These results demonstrate that, although the combination of PI3K inhibitor and rapamycin is more effective in inhibiting proliferation of AML, the concomitant inhibition of PI3K and mTOR by LY 294002 and rapamycin has more inhibitory effects on ATRA-mediated differentiation

than the presence of PI3K-inhibitor alone, and diminishes positive effects of rapamycin on leukemia cell differentiation.

**Ostojic A, Vrhovac R, Verstovsek S. Ruxolitinib: a new JAK1/2 inhibitor that offers promising options for treatment of myelofibrosis. *Future Oncol.* 2011 Sep;7(9):1035-43.**

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Ruxolitinib (INCB018424) is the first potent, selective, oral inhibitor of JAK1 and 2 being developed for clinical use. Its major cellular and systemic effects are proliferation inhibition, apoptosis induction and reduction in cytokine plasma levels, all mediated by the drug's inhibition of JAKs' ability to phosphorylate STAT. In initial clinical trials of its use in myelofibrosis, ruxolitinib exhibited durable efficacy in reduction of splenomegaly and alleviation of constitutional symptoms. Patients also showed weight gain and improvement in general physical condition. The dose-limiting toxicity was thrombocytopenia. In preliminary findings of a Phase III trial in patients with primary, postpolycythemia-*vera*, or postessential-thrombocytopenia myelofibrosis, administration at an initial dosage of 15 or 20 mg twice daily led to a spleen-volume response rate ( $\geq 35\%$  reduction at 24 weeks) of 41.9 versus 0.7% for placebo ( $p < 0.0001$ ); furthermore, 45.9% of the ruxolitinib recipients had  $\geq 50\%$  improvement in symptom score (on the modified Myelofibrosis Symptom Assessment Form version 2.0) versus 5.3% for placebo ( $p < 0.0001$ ). Ruxolitinib recipients also showed improvement in parameters of quality of life.

Radić J, Ljutić D, Radić M, Kovačić V, Dodig-Ćurković K, Sain M. Kidney Transplantation Improves Cognitive and Psychomotor Functions in Adult Hemodialysis Patients. *Am J Nephrol.* 2011 Sep 16;34(5):399-406.

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Background: Kidney failure is believed to have a negative impact on cognitive function, and cognitive impairment is common among maintenance hemodialysis (HD) patients. Previous studies have shown a beneficial effect of kidney transplantation in certain cognitive tests but not across all cognitive domains assessed. But, most of these studies performed a cross-sectional analysis, suffered from lack of standardization of adequate dialysis dose, hemoglobin level, and insufficient sensitivity of neuropsychological tests. The aim of this study was to evaluate the effect of successful kidney transplantation on cognitive and psychomotor function in adequately dialyzed HD patients without severe anemia, using sensitive neuropsychological tests. Methods: Twenty-one medically stable patients (aged  $45.1 \pm 7.9$  years) on maintenance HD ( $7.6 \pm 4.2$  years) were investigated before and  $20.5 \pm 8.5$  months after successful kidney transplantation using Complex Reactimeter Drenovac, a battery of computer-generated psychological tests which measure a simple visual discrimination of signal location, short-term memory, simple convergent visual orientation and convergent thinking. Results: Our findings indicated significantly better cognitive and psychomotor performance after transplantation on tests that assess processing speed, attention, short time memory, convergent thinking and executive functioning. Also, significant negative correlation between follow-up time after transplantation and cognitive and psychomotor performance in minimum time of solving test of convergent thinking was found. Conclusion: We conclude that cognitive and psychomotor functions are superior after successful kidney transplantation compared with HD, and that early beneficial effects of transplantation are not transient and cognitive and psychomotor performance might be even improved in time following successful transplantation.

Pećin I, Cvorišćec D, Miletić-Medved M, Dika Z, Cvitković A, Vitale K, Leko N, Novaković D, Sertić J, Kos J, Jelaković B. Angiotensin-Converting Enzyme Gene Polymorphism and N-Acetyl- $\beta$ -D-Glucosaminidase Excretion in Endemic Nephropathy. *Nephron Clin Pract.* 2011;119(2):c105-12.

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Background: Tubular proteinuria and enzymuria are hallmarks of endemic nephropathy (EN). The role of I/D angiotensin convertase (ACE) gene polymorphism has not yet been elucidated in this peculiar chronic tubulointerstitial nephritis, and our aim was to investigate the role of this polymorphism in EN focusing on the urinary N-acetyl- $\beta$ -D-glucosaminidase (NAG) excretion, a biomarker of proximal tubular damage. Methods: ACE genotype and allele frequencies were determined in 229 farmers (147 women and 82 men) from an endemic Croatian village. The farmers were stratified according to the WHO criteria into the following subgroups: those 'at risk' for EN ( $n = 37$ ), 'suspected of having EN' ( $n = 57$ ), and 'others' ( $n = 135$ ). Results: There were 74 (32.3%) subjects homozygous for the D allele, 99 (43.2%) heterozygous (ID genotype) and 56 (24.4%) homozygous for the I allele. No differences in allele frequency were found between the established WHO subgroups ( $p > 0.05$ ). In the whole group, DD subjects had significantly higher values of diastolic blood pressure ( $p = 0.003$ ) and urinary NAG than subjects with ID and II genotype ( $5.5 \pm 1.2$  vs.  $4.0 \pm 3.0$  vs.  $3.8 \pm 4.2$ , respectively;  $p = 0.023$ ). The highest values of serum creatinine ( $p = 0.02$ ), proteinuria ( $p = 0.03$ ) and urinary NAG ( $6.0 \pm 3.7$  vs.  $3.7 \pm 2.1$  vs.  $3.0 \pm 1.6$ , respectively;  $p = 0.008$ ) were observed in those suspected of having EN group with the DD genotype. Conclusion: ACE gene polymorphism is not a risk factor for EN. However, it might influence the clinical course of EN, and increased excretion of NAG might be a prognostic marker of this chronic tubulointerstitial nephritis.

Petanjek Z, Judaš M, Šimic G, Rasin MR, Uylings HB, Rakic P, Kostovic I. Extraordinary neoteny of synaptic spines in the human prefrontal cortex. *Proc Natl Acad Sci U S A.* 2011 Aug 9;108(32):13281-6.

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The major mechanism for generating diversity of neuronal connections beyond their genetic determination is the activity-dependent stabilization and selective elimination of the initially overproduced synapses [Changeux JP, Danchin A (1976) *Nature* 264:705-712]. The largest number of supernumerary synapses has been recorded in the cerebral cortex of human and nonhuman primates. It is generally accepted that synaptic pruning in the cerebral

cortex, including prefrontal areas, occurs at puberty and is completed during early adolescence [Huttenlocher PR, et al. (1979) *Brain Res* 163:195-205]. In the present study we analyzed synaptic spine density on the dendrites of layer IIIc cortico-cortical and layer V cortico-subcortical projecting pyramidal neurons in a large sample of human prefrontal cortices in subjects ranging in age from newborn to 91 y. We confirm that dendritic spine density in childhood exceeds adult values by two- to threefold and begins to decrease during puberty. However, we also obtained evidence that overproduction and developmental remodeling, including substantial elimination of synaptic spines, continues beyond adolescence and throughout the third decade of life before stabilizing at the adult level. Such an extraordinarily long phase of developmental reorganization of cortical neuronal circuitry has implications for understanding the effect of environmental impact on the development of human cognitive and emotional capacities as well as the late onset of human-specific neuropsychiatric disorders.

**Pećina-Šlaus N, Nikuševa Martić T, Zeljko M, Bulat S. Brain metastases exhibit gross deletions of the APC gene. *Brain Tumor Pathol.* 2011 Jul;28(3):223-8.**

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Candidate genes involved in metastasis to the brain require investigation. In the present study, the adenomatous polyposis coli (APC) gene was analyzed in a set of human brain metastases. Gross deletions of the APC gene were tested by polymerase chain reaction/loss of heterozygosity (LOH) using the restriction fragment length polymorphism method performed by the use of MspI and RsaI genetic markers inside exon 15 and exon 11. Among 21 brain metastases analyzed, 58.8% of samples showed LOH of the APC gene. When assigning the genetic changes to a specific primary tumor type, 6 LOHs were found in metastases originated from lung and 4 LOHs in metastases from colon. The main effector of the wnt signaling, beta-catenin, was upregulated in 42.9% of cases and transferred to the nucleus in 28.6% of metastasis cases. Our findings suggest that genetic changes of the tumor suppressor gene APC, a component of the wnt pathway, represent a part of the brain metastasis genetic profile.

**Matak I, Bach-Rojecky L, Filipović B, Lacković Z. Behavioral and immunohistochemical evidence**

**for central antinociceptive activity of botulinum toxin A. *Neuroscience.* 2011 Jul 14;186:201-7.**

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Botulinum toxin A (BTX-A) is approved for treatment of different cholinergic hyperactivity disorders, and, recently, migraine headache. Although suggested to act only locally, novel observations demonstrated bilateral reduction of pain after unilateral toxin injection, and proposed retrograde axonal transport, presumably in sensory neurons. However, up to now, axonal transport of BTX-A from periphery to CNS was identified only in motoneurons, but with unknown significance. We assessed the effects of low doses of BTX-A injected into the rat whisker pad (3.5 U/kg) or into the sensory trigeminal ganglion (1 U/kg) on formalin-induced facial pain. Axonal transport was prevented by colchicine injection into the trigeminal ganglion (5 mM, 2 µl). To find the possible site of action of axonally transported BTX-A, we employed immunohistochemical labeling of BTX-A-truncated synaptosomal-associated protein 25 (SNAP-25) in medullary dorsal horn of trigeminal nucleus caudalis after toxin injection into the whisker pad. Both peripheral and intraganglionic BTX-A reduce phase II of formalin-induced pain. Antinociceptive effect of BTX-A was prevented completely by colchicine. BTX-A-truncated SNAP-25 in medullary dorsal horn (spinal trigeminal nucleus) was evident 3 days following the peripheral treatment, even with low dose applied (3.5 U/kg). Presented data provide the first evidence that axonal transport of BTX-A, obligatory for its antinociceptive effects, occurs via sensory neurons and is directed to sensory nociceptive nuclei in the CNS.

**Pavić I\*, Topić RZ, Raos M, Aberle N, Dodig S. Interferon-γ Release Assay for the Diagnosis of Latent Tuberculosis in Children Younger Than 5 Years of Age. *Pediatr Infect Dis J.* 2011 Oct;30(10):866-70.**

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Background: There are limited data available on interferon-γ release assay (IGRA) performance in children up to 5 years of age, with documented exposure to active tuberculosis (TB). The aim of this study was to evaluate (1) the influence of infectivity of adult source cases on test results, (2) the impact of age, and (3) the level of agreement, between

IGRA and tuberculin skin test (TST) results. Methods: A total of 142 Bacille Calmette-Guerin-vaccinated children up to 5 years of age were investigated because of a history of exposure to active TB. QuantiFERON-TB Gold In-Tube IGRA (QFT) and TST assays were performed. Results: Test results were significantly influenced by positive finding of cavitory lesions (QFT, odds ratio [OR] = 6.15; TST, OR = 7.48) and positive acid-fast bacilli (QFT, OR = 4.01; TST, OR = 4.47) in active TB contacts. QFT resulted in 1 indeterminate response (0.7%), attributable to low mitogen. There was no evidence for age having any effect on QFT performance. The 2 tests showed a moderate overall concordance (89%;  $\kappa = 0.591$ ) at a TST cutoff value of  $\geq 10$  mm. Conclusions: Association

of positive QFT and TST results with risk factors for infection in child contacts (presence of cavitory lesions and acid-fast bacilli smear positivity in index cases) suggests that both the tests have good diagnostic accuracy. However, there was significant discord between results of the 2 tests that could not be definitively resolved. Thus, in a high-risk population of children up to 5 years of age, both tests (QFT and TST) should be performed and the child should be considered infected if either or both tests are positive.