

## CROATIAN INTERNATIONAL PUBLICATIONS

Rahighi S, Ikeda F, Kawasaki M, Akutsu M, Suzuki N, Kato R, Kensch T, Uejima T, Bloor S, Komander D, Randow F, Wakatsuki S, Đikić I\*. **Specific recognition of linear ubiquitin chains by NEMO is important for NF-kappaB activation.** *Cell.* 2009;136:1098-109.

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Activation of nuclear factor-kappaB (NF-kappaB), a key mediator of inducible transcription in immunity, requires binding of NF-kappaB essential modulator (NEMO) to ubiquitinated substrates. Here, we report that the UBAN (ubiquitin binding in ABIN and NEMO) motif of NEMO selectively binds linear (head-to-tail) ubiquitin chains. Crystal structures of the UBAN motif revealed a parallel coiled-coil dimer that formed a heterotetrameric complex with two linear diubiquitin molecules. The UBAN dimer contacted all four ubiquitin moieties, and the integrity of each binding site was required for efficient NF-kappaB activation. Binding occurred via a surface on the proximal ubiquitin moiety and the canonical Ile44 surface on the distal one, thereby providing specificity for linear chain recognition. Residues of NEMO involved in binding linear ubiquitin chains are required for NF-kappaB activation by TNF-alpha and other agonists, providing an explanation for the detrimental effect of NEMO mutations in patients suffering from X-linked ectodermal dysplasia and immunodeficiency.

Kielczewska A, Pyzik M, Sun T, Krmpotić A\*, Lodoen MB, Munks MW, et al. **Ly49P recognition of cytomegalovirus-infected cells expressing H2-Dk and CMV-encoded m04 correlates with the NK cell antiviral response.** *J Exp Med.* 2009;206:515-23.

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Natural killer (NK) cells are crucial in resistance to certain viral infections, but the mechanisms used to recognize infected cells remain largely unknown. Here, we show that the activating Ly49P receptor recognizes cells

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infected with mouse cytomegalovirus (MCMV) by a process that requires the presence of H2-D(k) and the MCMV m04 protein. Using H2 chimeras between H2-D(b) and -D(k), we demonstrate that the H2-D(k) peptide-binding platform is required for Ly49P recognition. We identified m04 as a viral component necessary for recognition using a panel of MCMV-deletion mutant viruses and complementation of m04-deletion mutant (Deltam04) virus infection. MA/My mice, which express Ly49P and H2-D(k), are resistant to MCMV; however, infection with Deltam04 MCMV abrogates resistance. Depletion of NK cells in MA/My mice abrogates their resistance to wild-type MCMV infection, but does not significantly affect viral titers in mice infected with Deltam04 virus, implicating NK cells in host protection through m04-dependent recognition. These findings reveal a novel mechanism of major histocompatibility complex class I-restricted recognition of virally infected cells by an activating NK cell receptor.

Kaštelan D, Grubić Z, Kraljević I, Polašek O, Dušek T, Štingl K, et al. **The role of estrogen receptor-alpha gene TA polymorphism and aromatase gene TTTA polymorphism on peak bone mass attainment in males: is there an additive negative effect of certain allele combinations?** *J Bone Miner Metab.* 2009;27:198-204.

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Idiopathic osteoporosis in males is influenced predominantly by low peak bone mass as a feature under a strong genetic control. Among a number of candidate genes, alpha-estrogen receptor (ERalpha) and CYP19 genes are of particular interest due to important role of estrogen in pathophysiology of osteoporosis. In the present study we examined the association of certain allelic combinations of ERalpha gene thymine-adenine (TA) polymorphism and aromatase gene TTTA polymorphism on bone mineral density (BMD) in young men. The study sample consisted of 92 unrelated healthy male volunteers, aged 21-35. In each subject, lumbar spine and proximal femur BMD, parameters of bone turnover and 25-OHD level were measured.

Two ERalpha (TA)(n) alleles, allele 19 and allele 21, were found to be associated with lower BMD. The presence of allele 19 was associated with significantly lower lumbar spine (P = 0.006) and trochanter (P = 0.02) BMD while the subjects positive for allele 21 had significantly lower lumbar spine (P = 0.04), trochanter (P = 0.02) and total hip (P = 0.03) BMD. Men with CYP19 (TTTA)(7-3)/ERalpha (TA)(19) allele combination had significantly lower lumbar spine BMD (P = 0.02) and those with CYP19 (TTTA)(7-3)/ERalpha (TA)(21) allele combination had significantly lower BMD for all three measurements, i.e. lumbar spine (P = 0.02), femoral neck (P = 0.02) and total hip (P = 0.008). These particular combinations of high-risk alleles were associated with lower median lumbar spine, femoral neck and total hip BMD than either of the allele alone suggesting that negative effect of two risk alleles on peak bone mass add up.

**Grivennikov S, Karin E, Terzić J\*, Mucida D, Yu GY, Vallabhapurapu S, et al. IL-6 and Stat3 are required for survival of intestinal epithelial cells and development of colitis-associated cancer. *Cancer Cell*. 2009;15:103-13.**

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Colitis-associated cancer (CAC) is the most serious complication of inflammatory bowel disease. Proinflammatory cytokines have been suggested to regulate preneoplastic growth during CAC tumorigenesis. Interleukin 6 (IL-6) is a multifunctional NF-kappaB-regulated cytokine that acts on epithelial and immune cells. Using genetic tools, we now demonstrate that IL-6 is a critical tumor promoter during early CAC tumorigenesis. In addition to enhancing proliferation of tumor-initiating cells, IL-6 produced by lamina propria myeloid cells protects normal and premalignant intestinal epithelial cells (IECs) from apoptosis. The proliferative and survival effects of IL-6 are largely mediated by the transcription factor Stat3, whose IEC-specific ablation has profound impact on CAC tumorigenesis. Thus, the NF-kappaB-IL-6-Stat3 cascade is an important regulator of the proliferation and survival of tumor-initiating IECs.

**Gornik I, Wagner J, Gašparović V, Lauc G, Gornik O. Free serum DNA is an early predictor of severity in acute pancreatitis. *Clin Biochem*. 2009;42:38-43.**

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OBJECTIVES: Cell-free DNA has been investigated as a diagnostic marker in many diseases, including acute condi-

tions. Our hypothesis was that in acute pancreatitis free serum DNA correlates with the extent of pancreatic necrosis and that it may be an early marker of severity. DESIGN AND METHODS: Free DNA was measured in sera from 30 patients with acute pancreatitis at admission, on the first, fourth and seventh day following admission. RESULTS: On the first day following admission patients who would develop severe pancreatitis had significantly higher serum DNA levels than those with mild disease (median 0.271 ng/microL vs. 0.059 ng/microL respectively; P<0.001). This parameter showed very good characteristics as a potential severity predictor (area under ROC curve 0.97). Free serum DNA was in correlation with the extent of pancreatic necrosis. CONCLUSIONS: Free serum DNA correlates with the extent of pancreatic necrosis and is a potential early marker of severe acute pancreatitis.

**Puljak L, Kojundžić SL, Hogan QH, Sapunar D. Lidocaine injection into the rat dorsal root ganglion causes neuroinflammation. *Anesth Analg*. 2009;108:1021-6.**

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BACKGROUND: Injury of a spinal nerve or dorsal root ganglion (DRG) during selective spinal nerve blocks is a potentially serious complication that has not been adequately investigated. Our hypothesis was that local anesthetic injection into these structures may result in an inflammatory response and hyperalgesia. METHODS: We evaluated inflammatory and behavioral responses after injection of 4 microL lidocaine or saline into the L5 spinal nerve or DRG of rats after partial laminectomy. Behavioral testing was performed before and after surgery to examine hyperalgesia in response to nociceptive mechanical stimulation of the foot. DRGs were harvested and stained, and rings of immunoreactive glial cells around neurons were counted. RESULTS: Animals demonstrated hyperalgesia on the ipsilateral paw up to 4 days after lidocaine injection into the DRG but not after injection into the spinal nerve. The number of glial fibrillary acid protein immunopositive glial cell rings, which represent activation of satellite cells, significantly increased in DRGs after injection of lidocaine into either the DRG or the spinal nerve. The number of glial fibrillary acid protein-positive cells in the lidocaine-injected group was significantly larger than in the saline-injected group. Sporadic OX-42 immunopositive cells, which represent activated microglia, were also seen in lidocaine-injected DRGs. Testing for Pan-T expression, which labels activated T lymphocytes, showed no positive

cells. CONCLUSIONS: Lidocaine injection into the DRG may produce hyperalgesia, possibly due to activation of resident satellite glial cells. In a clinical setting, local anesthetic injection into the DRG should be avoided during selective spinal nerve blocks.

**Bičanić G, Delimar D, Delimar M, Pećina M. Influence of the acetabular cup position on hip load during arthroplasty in hip dysplasia. *Int Orthop.* 2009;33:397-402.**

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Placement of the acetabular cup during total hip arthroplasty is of great importance because usually every deviation from the ideal centre of rotation negatively influences endoprosthesis survival, polyethylene wear and hip load. Here we present hip load change in respect to various acetabular cup positions in female patients who underwent total hip replacement surgery due to hip dysplasia. The calculation suggests that, in the majority of cases, for every millimeter of lateral displacement of the acetabular cup (relative to the ideal centre of rotation) an increase of 0.7% in hip load should be expected and for every millimeter of proximal displacement an increase of 0.1% in hip load should be expected (or decreased if displacement is medial or distal). Also, for every millimeter of neck length increase, 1% decrease is expected and for every millimeter of lateral offset, 0.8% decrease is expected. Altogether, hip load decreases when the cup is placed more medially or distally and when the femoral neck is longer or lateral offset is used.

**Brizić I, Modun D, Vuković J, Budimir D, Katalinić V, Boban M. Differences in vasodilatory response to red wine in rat and guinea pig aorta. *J Cardiovasc Pharmacol.* 2009;53:116-20.**

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We examined and compared mechanisms of the red wine (RW)-induced vasorelaxation in guinea pig (GP) and rat aorta. Acetylcholine-induced relaxation of norepinephrine-precontracted aortic rings was stronger in rat aorta than in GP aorta, whereas RW-induced vasorelaxation was stronger in GP aorta. L-nitro-arginine methyl ester (L-NAME) abolished RW-induced vasorelaxation in rat aorta, whereas in GP aorta, it was only reduced by 50%. To examine

mechanisms of the L-NAME-resistant relaxation, GP aortic rings were additionally exposed to indomethacin, clotrimazole, and their combination. Indomethacin insignificantly reduced RW-induced relaxation, but in combination with L-NAME, the relaxation was synergistically decreased (80%). After clotrimazole exposure, the relaxation was reduced by 25%, and addition of indomethacin caused no further reduction. Only the combination of L-NAME, indomethacin, and clotrimazole prevented RW-induced vasorelaxation. RW-induced vasorelaxation in KCl-precontracted GP rings was significantly smaller ( $E_{max}$  78.31%  $\pm$  6.09%) than the RW-induced relaxation in norepinephrine-precontracted rings ( $E_{max}$  126.01%  $\pm$  2.11%). L-NAME in KCl-precontracted GP rings prevented RW-induced vasorelaxation. In conclusion, different pathways are involved in the RW-induced vasorelaxation in GP aorta, in contrast to rat aorta, in which NO plays main role. Therefore, the uncritical extrapolation of the results from one species to another could be misleading.

**Dorević G, Matusan-Ilijaš K, Babarović E, Hadžisejdić I, Grahovac M, Grahovac B, et al. Hypoxia inducible factor-1alpha correlates with vascular endothelial growth factor A and C indicating worse prognosis in clear cell renal cell carcinoma. *J Exp Clin Cancer Res.* 2009;28:40.**

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BACKGROUND: The role of angiogenesis in the pathogenesis of renal cell carcinoma is well recognized, however, the influence of tumor cells in this activity has not yet been fully clarified. The aim of this study was to analyze the expression of hypoxia inducible factor-1alpha (HIF-1alpha), a regulatory factor of angiogenic switch, in comparison to vascular endothelial growth factor A and C (VEGF-A and VEGF-C), recognized to be involved in blood and lymph vessel neoangiogenesis, with potential association in the prognosis of patients with renal cell carcinoma. METHODS: Ninety-four patients with diagnosis of clear cell renal cell carcinomas (CCRCC), all clinicopathological characteristics and overall survival were unrolled in this study. Immunohistochemically VEGF-A, VEGF-C, HIF-1alpha and Ki67 were detected on tumor cells and the staining was performed on tissue microarrays (TMA). The staining was evaluated as a percentage of cytoplasmic or nuclear positive tumor cells. RESULTS: Variable expression of all three proteins was confirmed. Both angiogenic factors demonstrated perimembranous or diffuse cytoplasmic staining, with diffuse pattern positively associated ( $p < 0.001$ ). Nuclear HIF-1al-

pha expression (nHIF-1alpha) showed inverse correlation with diffuse cytoplasmic VEGF-A ( $p = 0.002$ ) and VEGF-C ( $p = 0.053$ ), while cytoplasmic HIF-1alpha expression (cHIF-1alpha) showed positive correlation with diffuse staining of both angiogenic factors ( $p < 0.001$ ;  $p < 0.001$ , respectively). In comparison to clinicopathological characteristics, a higher nuclear grade ( $p = 0.006$ ;  $p < 0.001$ , respectively), larger tumor size ( $p = 0.009$ ;  $p = 0.015$ , respectively), higher stage ( $p = 0.023$ ;  $p = 0.027$ , respectively) and shorter survival ( $p = 0.018$ ;  $p = 0.024$ , respectively) were associated with overexpression of cHIF-1alpha and diffuse cytoplasmic VEGF-A expression. In contrary, overexpression of nHIF-1alpha was associated with better diagnostic parameters i.e. lower nuclear grade ( $p = 0.006$ ), smaller tumor size ( $p = 0.057$ ), and longer survival ( $p = 0.005$ ). **CONCLUSION:** Overexpression of VEGF-A and cHIF-1alpha in tumor cells highlights a more aggressive subtype of CCRCC that might have some clinical implications. The significance of nHIF-1alpha expression associated with better differentiated tumors should be further elucidated.

**Škerk V, Markovinović L, Zekan S, Jakšić J, Židovec Lepej S, Markotić A, et al. The significance of Chlamydia trachomatis in urethritis and prostatitis - differences in therapeutic approach – Croatian experience. J Chemother. 2009;21:63-7.**

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We examined a total of 1014 patients over 18 years of age; 252 with urethritis and 762 with chronic prostatitis syndrome. the mean age of patients with urethritis was 32.7 and with prostatitis syndrome 37.6 years. Clinical symptoms of urethritis were present from a few days to several months. in patients with chronic prostatitis syndrome, symptoms were present for at least 3 months. Chlamydia trachomatis alone was confirmed in 26 (10%) and in combination with Ureaplasma urealyticum in 6 (2%) patients with urethritis. in 171 (68%) patients with urethritis neither C. trachomatis nor U. urealyticum or Mycoplasma hominis were found. C. trachomatis alone was confirmed in 70 (9%), and in combination with other microorganisms in 7 (1%) patients with chronic prostatitis syndrome. in Croatia,

the frequency of chronic chlamydial prostatitis has not significantly changed in the last 10 years, while the frequency of infections among adolescents decreased. the recommended regimen for acute chlamydial urethritis in Croatia is azithromycin 1.0 g as a single dose, and a total dose of 4-4.5 g azithromycin for chronic chlamydial prostatitis.

**Glavaš D, Baković D, Obad A, Palada I, Brešković T, Valić Z, et al. Effects of tetrahydrobiopterin on venous bubble grade and acute diving-induced changes in cardiovascular function. Clin Physiol Funct Imaging. 2009;29:100-7.**

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**INTRODUCTION:** Self-contained underwater breathing apparatus diving reduces cardiovascular function and increases pulmonary artery pressure (PAP) up to 3 days after a single dive. Acute antioxidants partially attenuated arterial endothelial dysfunction, whereas cardiac and PA functions were unaffected. We tested the hypothesis that acute tetrahydrobiopterin (BH(4)), as a cofactor of endothelial nitric oxide (NO) synthase, reduces bubble grade (BG) and attenuates alteration in cardiovascular function after diving because of increased NO bioavailability. **MATERIALS AND METHODS:** Mean PAP (mPAP), PA acceleration time and right ventricle ejection time, left ventricle ejection fraction (LV-EF) and BG were measured after oral placebo (P), vitamin C (C) or a combination of vitamin C and BH(4) (BH(4)) in a randomized, placebo controlled trial before and after field dive to 30 m of sea water for 30 min bottom time. **RESULTS:** Eight recreational divers performed three dives with a 3-days period between them. Regarding the primary hypothesis, no difference was observed between post-dive changes in BG ( $2.1 \pm 2.2$  bubbles  $\text{cm}^{-2}$ ) for P,  $3.4 \pm 3.9$  for C and  $3.6 \pm 2.1$  for BH(4)), mPAP ( $25.6 \pm 6.5$  mmHg for P,  $25.9 \pm 8.6$  for C and  $22.6 \pm 3.5$  for BH(4)) and LV-EF ( $62.6 \pm 4.6\%$  for P,  $61.4 \pm 3.9$  for C and  $61.6 \pm 3.7$  for BH(4)) with all three conditions. **CONCLUSION:** This suggests that co-administration of BH(4) and vitamin C does not improve heart and pulmonary artery function after diving.



