PS021

Regulation of transcription factor MEF2C by RNA binding protein HuR

Z. Anyu 1, G. Shi 1, A. Xie 1, D. Aksoy 2*, S. Dudley 1

1 Cardiovascular Research Center, The Warren Albert Medical School of Brown University, Providence Rhode Island, United States
2 Marmara University School of Medicine, Istanbul, Turkey

E-mail address: sdiilsadaksoy@gmail.com
(D. Aksoy).

Aim: We hypothesized that HuR RNA binding protein regulates MEF2C expression through association with MEF2C mRNA.

Introduction: MEF2C is earliest expressed member of the MADS-box super family during heart development. In the postnatal heart, decreased expression of MEF2C has been associated with myotonic dystrophy type 1 (DM1) heart disease. Hu proteins are known to regulate a wide range of gene expression by modulating mRNA’s half-lives.

Methods: We use Human Fetal Cardiomyocyte cell line RL14. Cells are transfected with Superperfect Transfection Reagent(Qiagen). And RNA Isolation performed by using RNAeasy Plus Mini Kit. Real Time quantitative PCR (q–PCR) analysis performed using Fast SYBR Green Master Mix.

Results: Over expression of HuR in cardiomyocytes derived from primary human fetal ventricle increased MEF2C mRNA 47.3% (p = 0.01). Knocking down of HuR by siRNA decreased MEF2C mRNA by 62% (p = 0.01). RNA Immunoprecipitation showed HuR associated with MEF2C mRNA.

Conclusion: Our results suggest that RNA binding protein HuR associates with MEF2C mRNA in cardiomyocytes. And also HuR positively regulates MEF2C mRNA expression.

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PS024

The effect of prenatal Vitamin C deficiency on endochondral ossification in guinea pigs

N. Rakočević

Medical Faculty, University of Novi Sad, Serbia
E-mail address: rakocевичnatali@gmail.com.

Aim: The aim of the research is to investigate the effect of prenatal vitamin C deficiency on endochondral ossification in guinea pigs.

Introduction: Vitamin C is an essential nutrient which inter alia enables the synthesis of collagen and therefore endochondral ossification. Throughout years a lot of research has been published investigating the exact role of vitamin C and the impairment developed due to its deficiency. However there is insufficient data about the effect of prenatal deficit of vitamin C on the developing bone structures.

Methods: The study encompassed 14 fertilized female albino guinea pigs. Their diet was comprised of vitamin C-free food and ad libitum water enriched with vitamin C. The 10th day of fertilization, experimental group was depleted of vitamin C. Deprivation lasted until the 50th day, after which the females were sacrificed and their fetuses were taken out. Forelegs of fetuses were fixed and dehydrated, after which they were embedded in paraffin and

References


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longitudinal sections were made. The stain used for histology was Alcin&Alizarin.

**Results:** The development of long bones in vitamin C deficient guinea pigs is considerably stagnant. Hyaline cartilage models are significantly shortened. Ossification in the diaphyses of carpal and metacarpal bones are absent, and the organization of the epiphysial plates is very irregular with the reduction of number of chondrocytes. Moreover, there are numerous haemorrhagic regions and subperichondrial bleeding with separation of perichondrium.

**Conclusion:** Deprivation of vitamin C during inrauterine period disables normal development of long bones. Disorder of hyaline cartilage models was seen, as well as the disorder of ossification.

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**PS060**

**Peculiarities of expression of apoptosis markers in the tissues of primary fallopian tubes carcinoma**

Franklin Unawunwa*, Natalia Hyriavenko, Anna Korobchanska, Mykola Lyndin, Vladyslav Sikora

*Sunny State University
E-mail address: unawunwafranklin@yahoo.com (F. Unawunwa).

**Aim:** immunohistochemical analysis of apoptosis markers in the tissue of PFTC.

**Introduction:** Primary fallopian tubes carcinoma is a rare case among oncological diseases of female genital organs, but the mortality rate is rather high. Nowadays, the prognostic factors of this neoplasia are not fully determined. The data on the p53 and bcl2 proteins expression and their use as prognostic factors in patients with malignant tumors of many locations are contradictory.

**Methods:** the study was conducted on 66 samples of fallopian tubes tumor tissue. To study the apoptosis peculiarities of tumor cells the mouse monoclonal antibodies for bcl-2 (clone 100/D5) and p53 (clone SP5) were used. Mathematic calculations were done using Microsoft Excel 2010 with AtteStat 12.0.5.

**Results:** The high expression of p53 was found in patients of all clinical stages. Mutations of p53 increased with spreading of the neoplastic process. Strong correlation of p53 presence in tumor samples and clinical stage of the disease was determined ($r = 0.77$). In contrast to the abovementioned protein the study of bcl-2 showed the moderate negative correlation between this protein and the stage of the disease ($r = -0.54$). Analysis of the dependence of p53 expression with the presence or absence of lymph nodes metastasis showed a direct correlation between the indicators ($r = 0.25$). Thus the level of p53 expression in patients with N1 was $80.6 \pm 2.7\%$ compared with the N0 group ($29.7 \pm 3.6\%$). The stage of neoplasia differentiation is in moderate direct correlation with p53 expression ($r = 0.58$) and in inverse with bcl-2 ($r = -0.64$).

**Conclusion:** Expression of p53 depends on neoplasia spreading and stage of tumor differentiation. The expression of p53 is an independent prognostic marker for N-status and helps to classify the patients into “risk” groups.

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**PS064**

**Analysis of combined impact of doxorubicin and menadione on human leukemia Jurkat T cells**

Alexandru Ionut Duta*, Ioana Teodora Tofolean, Ramona Madalina Babes, Constanta Ganea, Irina Baran

“Carol Davila” University of Medicine and Pharmacy, Department of Biophysics, Bucharest, Romania
E-mail address: alexxxduta@yahoo.com (A.I. Duta).

**Aim:** The anti-proliferative effect and the mechanism of action of doxorubicin(DOX) in combination with menadione(MD) were studied in Jurkat T cells, a model for acute lymphoblastic leukaemia (ALL).

**Introduction:** Doxorubicin is a well-characterized and successful antineoplastic drug commonly used in various cancer treatments, including ALL. Menadione has proven a strong pro-apoptotic effect in Jurkat cells.1-3

**Methods:** Cell cycle, apoptosis/necrosis and the oxidative status were assessed by flow cytometry on propidium ioidide, Annexin V–FITC/PI and CM-H2DCFDA/7-AAD labelled cells, respectively.

**Results:** Oxidative stress induced within 4h by MD (IC50 = 11.5 μM) was reduced in the presence of 500 nM DOX (IC50 = 22.0 μM). After treatments of 18 h, DOX induced cell cycle arrest displaying a trimodal distribution; successive G2/M, S and G0/G1 blockage was produced with an IC50 of 49 nM, 464 nM and 1866 nM, respectively, whereas in the presence of 7.5 μM MD, increasing levels of DOX mainly induced S-phase arrest. Within 18 hours of exposure, DOX induced apoptosis in a biphasic dose–dependent manner ($K_d = 335$ nM and 3.29 μM, respectively). Addition of 7.5 μM MD enhanced apoptosis at <300 nM DOX, but reduced cell death at higher levels of DOX. However, 48 h after drug removal the apoptotic rate was considerably higher in cells exposed to DOX:MD, which also showed consistent fractions of early apoptosis (up to 44%). The efficacy of DOX was doubled by MD ($K_d = 46.5$ nM in the presence, and $K_d = 99$ nM and 143 nM in the absence of MD).

**Conclusion:** Data indicate that clinically relevant levels of MD and DOX in combined treatments can exert considerable cytotoxic impact on Jurkat cells, via cell cycle arrest and apoptosis induction. These findings could encourage new therapeutic strategies to improve the therapeutic index of doxorubicin in ALL treatments.

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**PS068**

**Effect of symptom interval and demographic characteristics on initial stage of malignant tumors in children**

R. Grujićic*, O. Djurmez, M. Trkulja, J. Lazić, M. Bjelić

School of Medicine, University of Belgrade, Serbia