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Aim: To develop an experimental platform for *in vivo* investigation of candidate genetic modifiers of somatic CAG instability in Huntington's disease.

Introduction: Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by a CAG repeat expansion within the huntingtin gene (*HTT*).¹ Despite being a monogenic disorder, for which the mutation has been known for some time now, no cure or disease-modifying therapy is available, indicating that novel approaches are critical.

Somatic CAG repeat instability, characteristic of mutant *HTT* alleles, is inversely correlated with patient age of onset and may contribute to HD pathogenesis.^{2,3} This phenotype, common to other trinucleotide repeat disorders,⁴ was previously shown to be DNA mismatch repair (MMR) dependent.⁵ The DNA repair machinery was further implicated as a modifier of HD age of motor onset in a recent genome wide association study, underlining its promise as a relevant disease mechanism that could potentially be therapeutically targeted.⁶

In this study, we are developing a CRISPR/Cas9-based approach that will enable the investigation of candidate genetic modifiers of HD age of onset as potential modifiers of somatic CAG repeat instability in a HD mouse model.

Methods: We have developed CRISPR reagents against known and candidate genetic modifiers of somatic CAG instability in Huntington's disease. In preliminary experiments, we treated HD mice with CRISPR reagents against *Mlh1* and investigated the level of gene editing achieved as well as the impact on liver CAG instability.

Results: We were able to significantly suppress the CAG expansion process in the liver of HD mice by knocking out the *Mlh1* gene in our *in vivo* CRISPR platform. The efficiency achieved in modifying the instability phenotype makes us very confident that we will be able to test and validate additional candidate modifiers. To that end, we have already validated reagents for efficient knockout of a subset of known and candidate modifier genes and we have developed assays that will allow detailed characterization of gene editing at these sites.

Conclusion: We have successfully developed an *in vivo* CRISPR-Cas9-based platform that allows for knocking out genes of interest in the liver of adult mice, and consequently perturb the somatic CAG expansion process. We will next use this tool to test the role that candidate genes might play in that disease-relevant process. While the scope of this project was liver oriented, future work will also be aimed at targeting the striatum which is the main site of HD-related pathology.

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Oncology & Molecular Biology Poster Session
Sunday, September 17th, 10h00

PS021

Regulation of transcription factor MEF2C by RNA binding protein HuR



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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 Superfect Transfection Reagent(Qiagen). And RNA Isolation performed by using RNeasy 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



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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

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

Results: The development of long bones in vitamin C deficient guinea pigs are considerably stagnant. Hyaline cartilage models are significantly shortened. Ossification in the diaphyses of carpal and metacarpal bones are absent, and the organization of the epiphyseal 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



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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 leukaemia Jurkat T cells



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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 proapoptotic effect in Jurkat cells.¹⁻³

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

Results: Oxidative stress induced within 4h by MD ($IC_{50}=11.5 \mu M$) was reduced in the presence of 500 nM DOX ($IC_{50}=22.0 \mu M$). 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 IC_{50} 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



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