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AB0172

PGC-1A REGULATES AUTOPHAGY TO PROMOTE FIBROBLAST ACTIVATION AND TISSUE FIBROSIS

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Background: Peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α) is the best studied member of the family of coactivators. PGC-1α was initially identified through its interaction with PPARγ in brown adipose tissue. Recent evidence further indicates that PGC-1α may also modulate the transcription of autophagy-related genes, which has recently been shown to be required for fibroblast-to-myofibroblast differentiation under fibrotic conditions. However, the role of PGC-1α in the pathogenesis of SSc has not been investigated.

Objectives: The aim of the present study was to evaluate the role of the coactivator PGC-1α on autophagy and to evaluate its role in the pathologic activation of fibroblasts in SSc.

Methods: Expression of PGC-1α was analyzed by RT-PCR, Western blot and immunofluorescence. Modulation of autophagy was analyzed by reporter studies by expression of autophagy related genes. The effects of PGC-1α knockdown on collagen production and myofibroblast differentiation were analyzed in cultured human fibroblasts and in two mouse models with fibroblast-specific knockout of PGC-1α.

Results: PGC-1α overexpression was detected by immunohistochemistry in skin sections of SSc patients and in experimental fibrotic murine skin, particularly in fibroblasts. Knockdown of PGC-1α inhibited the stimulatory effects of TGFβ on fibroblast activation with impaired induction of collagen as compared to control fibroblasts. Fibroblasts specific knockout of PGC-1α ameliorates experimental fibrosis in bleomycin-induced and adTBR-induced murine dermal fibrosis with decreased dermal thickness, hydroxyproline and myofibroblast counts compared to wild-type fibrotic mice. Incubation of dermal fibroblasts with TGFβ activated autophagy in control fibroblasts with increased expression of the autophagy-related genes ATG7 and BECLIN-1, enhanced conversion of LC3 I to LC3 II and decreased ratios of ILC3 I EGFP to LC3 II RFP in LC3 reporter assays. The expression levels of ATG7, BECLIN-1 and ILC3 II of TGFβ-stimulated PGC-1α knockout fibroblasts decreased compared to TGFβ stimulated wild-type fibroblasts. The ratio of ILC3 I EGFP to LC3 II RFP of TGFβ-stimulated PGC-1α knockout fibroblasts in reporter assays were comparable to unstimulated fibroblasts.

Conclusion: PGC-1α is upregulated in SSc and promotes autophagy to foster TGFβ-induced fibroblast activation. Targeting of PGC-1α prevents aberrant autophagy, inhibits fibroblast activation and tissue fibrosis.

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10. Basic and translational science in paediatric rheumatology

AB0173

ALLELIC POLYMORPHISM OF PROINFLAMMATORY CYTOKINE GENES AS A BASIS FOR THE FORMATION OF PHENOTYPES OF JUVENILE IDIOPATHIC ARTHRITIS

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Background: The pathological process of juvenile idiopathic arthritis (JIA) largely depends on pro-inflammatory cytokines, the polymorphism of the alleles of some genes of which we have the opportunity to study. No studies have been conducted on the dependence of certain features of the pathological process of JIA on the polymorphism of the IL-6(G-174C) and TNF(G308A) genes.

Objectives: To reveal the dependence of JIA phenotypes and its course on genetic polymorphism of alleles IL-6 and TNF.

Methods: Polymorphism of the IL-6 and TNF genes was studied by PCR-method using allele-specific primers 44 patients 1-17 y.o. (24f, 20m) with JIA. The level of IL-6 and TNFα in the serum was determined using ECLIA and CLIA methods.

Results: There were 73% cases with an unfavorable course of the disease (UCD) of the patients with the CC allele of the IL-6 gene, for most patients average activity was JADAS27 13.5±1.6. oJIA (50%) & uveitis (30%) were the most frequent among subgroups. The level of serum IL6 was 74.1±69.5 pg/ml, TNFα 27.4±17.3 pg/ml (ratio IL6/TNFα=4.3±2.1). Among patients with GC IL6 70% female, 79% with UCD. More often pJIA (36%, including all RF+) and eJIA (35%) were noted with the largest frequency of inclusion of the hip joints (33%), spine (35%), detection of secondary osteoporosis (43%). The metabolic changes were registered on the ECG in 82% cases. The serum IL-6 level was 11.35±2.95 pg/ml, TNF 24.75 pg/ml (IL-6/TNFα=0.047, p<0.05 vs CC allele). Children with GG IL-6 (wild allele) with a more favorable course of the JIA (31%, less than in the CC and GC groups (p<0.05), only 8% had the highest disease activity), the largest number of patients with sJIA (25%) was registered in this group. The detection of HLA B27 was significantly lower (p<0.05) than in other alleles, while 60% cases were ANA+ (more than in the group GC, p<0.05). The highest level of serum IL6 (35.3±18.9 pg/ml) & the highest average number of mutations in folate metabolism genes (4±0.51) were revealed in this group. The wild allele GG prevailed (n=32) among the TNF gene alleles, sex ratio 1:1, UCD in 70%. The number of active joints, ESR, CRP, ANA-positivity (50%), HLA B27+ (53%) were insignificantly higher than in GA TNF allele, while serum IL6 level (22.8±9.8 pg/ml) & TNFα (12.3±4.1 pg/ml) were lower. In patients with the GA TNF gene allele, an UCD (73%), eJIA (36%) were noted slightly more often. By such parameters as the patient's gender, the presence of uveitis, damage to the hip joints, the type of synovitis, metabolic changes on the ECG, indicators were observed comparable with the wild allele group. IL6 level was 48.3±39.2 pg/ml, TNFα 36.5±420.1 pg/ml, IL6/TNFα=0.07±0.06 (vs 1.9±0.5 in GG group, p<0.05). The genotype of two wild alleles TNF GG with IL6 GC expectedly showed the smallest proportion of the UCD (33%, p<0.05), the most frequency of ANA-positivity (71%), with no uveitis and RF+pJIA in this group. All cases of RF+pJIA had TNF GA and IL6 GC. oJIA prevailed (57%) in the TNF GG&IL6 CC group, there was not a single case of sJIA, and the AJ number was the smallest (2.86±0.5). The largest group was TNF GG & IL6 GC (n=14). 91% of cases had UCD, AJ=6.6±2.4, damage to the hip joints in 40%, ESR 23.7±6.7 mm/h, CRP 14.5±5.4 mg/l, metabolic changes on the ECG in 100%, but ANA+ only at 13%. In general, there was no correlation between the cytokine content in the blood serum during of active disease in the examined children with features of allelic polymorphism of these genes.

Conclusion: Depending on the allele polymorphism of the IL-6 and TNF genes, certain phenotypes of the JIA course may be distinguished. Thus, revealing the polymorphism of these alleles in patients at the onset of the disease, we can predict to some extent its course and take this into account when choosing treatment tactics.

Disclosure of Interests: None declared

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AB0174

T REGULATORY CELLS LEVEL IN PERIPHERAL BLOOD OF PATIENTS WITH JUVENILE IDIOPATHIC ARTHRITIS AND ITS RELATION WITH DISEASE ACTIVITY

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Background: The most important T-cell subtype in maintenance of immune tolerance is T regulatory cells (Treg). These are characterized by CD4 and CD25 receptors on surface, and by showing FoxP3 regulatory factor, which is necessary for maintaining the suppressive activity of Treg cells in peripheral blood (PB). Previous studies have studied Treg cells in PB and synovial fluid in patients with Juvenile Idiopathic Arthritis (JIA). However, there was insufficient evidence to draw robust conclusions about Treg implication in JIA, due to small sample size and variable results across studies. A deeper understanding of regulatory mechanism in JIA may increase comprehension on variability among JIA subtypes and may help to establish prognosis on the follow up.

Objectives: To analyze Treg cells level in PB of JIA patients and its relation with disease activity.

Methods: Descriptive, cross-sectional, observational study conducted in a regional reference centre for Pediatric Rheumatology. We included consecutive patients with JIA diagnosed by ILAR criteria. The primary variable was the Treg percentage in PB measured by flow cytometry. To assess JIA activity, we used disease activity indexes (JADAS10, 27, 71 – CRP/ESR and cJADAS), Wallace remission criteria, VAS disease activity by patient/parents and physicians, morning stiffness, multidimensional evaluation (JAMAR) and acute phase reactants (CRP and ESR). Assessment of long-term damage was evaluated with JADI. Association analyses among study variables and Treg levels were performed by Pearson's correlation coefficient and Mann Whitney's U test.

Results: Ongoing study, we present a preliminary analysis with first 50 JIA patients. Mean age (SD) was 11.3 yr (4.6), being females 60%. Most common JIA subtype was persistent oligoarticular (42%) followed by RFneg polyarticular (24%). 42% patients were treated by csDMARD and 46% by biological agents. Mean levels of CRP and ESR were 0.18 mg/dl (0.3) and 6.3 mm/hr (5.4), respectively. At the time of the study, 84% of patients were in remission (Wallace criteria). Mean of JADAS 27-VSG, JADAS 27-PCR and cJADAS were 3.6 (5.1), 3.7 (5.1), and 3.7 (5.5), respectively. Mean long-term damage scores were 0.48 (1.1) for JADI-A and 0 for JADI-E. Mean levels of Treg cells in PB were 2.11% (1.1). The table shows the association between clinical variables and % of Treg. We can observe a significant, inverse and moderate correlation between Treg levels and disease activity by patient/parents, disability and quality of life (global and the physical component). Close to statistical significance, we found inverse and moderate correlation between Treg cells and all JADAS scores, cJADAS, disease activity by physician and morning stiffness. There was no association between Treg and acute phase reactants. Furthermore, there were no differences in Treg cells in Wallace remission ($p=0.692$) and regarding use of conventional or biological DMARD ($p=0.984$ and $p=0.386$, respectively).

Conclusion: According to our preliminary data, higher levels of Treg cells in PB of patients with JIA could be related with lesser disease activity and better quality of life. Larger studies are needed to confirm whether this Treg-mediated regulatory mechanism can have prognostic implication JIA.

Variables	r	p value
CRP (mg/dl)	-0.24	0.099
ESR (mm/hour)	-0.15	0.314
VAS-disease activity (patient / parents)	-0.28	0.049
VAS-disease activity (physician)	-0.25	0.081
JADAS10 (VSG)	-0.27	0.064
JADAS27 (VSG)	-0.27	0.064
JADAS71 (VSG)	-0.27	0.064
JADAS10 (PCR)	-0.28	0.054
JADAS27 (PCR)	-0.28	0.054
JADAS71 (PCR)	-0.28	0.054
c JADAS10	-0.26	0.065
Disability (JAFA – JAMAR)	-0.34	0.021
Quality of life (PRQL – JAMAR)	-0.39	0.007
Quality of life physical (PRQLPh – JAMAR)	-0.46	0.001
Quality of life psychosocial (PRQLPs-JAMAR)	-0.21	0.156
Morning Stiffness	-0.27	0.066

Disclosure of Interests: None declared

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11. Basic and translational pain science

AB0175 INNOVATIVE PREPARATION OF CURCUMIN NANOPARTICLES TO IMPROVE ANTI-INFLAMMATORY EFFECT IN RHEUMATIC DISEASE

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Background: Curcumin (Cur) as a natural compound can be used in the wide spectrum of healthy functions and pharmacological activities [1-4]. It shows great promise for medication of various pro-inflammatory chronic illnesses [5]. In this study, we evaluate the ability of poly(lactide-co-glycolide)(PLGA) and different grades of PVA (polyvinyl alcohol) and lecithin as a drug delivery system for poorly soluble Cur

Objectives: The goal of this study was to prepare and characterize Cur encapsulated PLGA and different grades of PVA and lecithin as an efficient nanocarrier for improve anti-inflammatory effect in rheumatic disease

Methods: The PLGA nanospheres were formulated and then characterized for percent yield, encapsulation efficiency, surface morphology, and in vitro drug release profiles. At first, 6mg of Cur was added to the organic phase including 24mg of polymer dissolved in 5mL of dichloromethane to constitute 1:4 (drug-to-polymer) ratios. Then, a mixture of PVA-lecithin (at about 5 cc) was added to maintain the stability of double emulsion droplets. The emulsion was continuously stirred at 300 rpm for 24 hours (at temperature of 37.5 °C) to evaporate the solvent, leaving behind the colloidal suspension of the drug-encapsulated nanoparticle in aqueous phase. The encapsulation of Cur into PLGA was characterized by Fourier transform infrared spectroscopy (FT-IR) and Transmission electron microscopy (TEM).

Results: Our studies achieved the successful formation of smooth surface and spherical shape Cur encapsulated into PLGA nanoparticles by the TEM image confirmed. The particle size distribution demonstrated a range of 30nm to 100nm, with the mean particle size being 45nm. FTIR study implies successful loading of Cur into the nanoparticles. We show high drug-loading efficiency about $98 \pm 0.5\%$ for 6% of Cur weight in total ingredients weight of PLGA (w/w). It was also seen that a slower sustained release of 10% CUR in 48 hours is observed with biocompatible PLGA in phosphate buffered saline (pH = 7.4). The MTT assay of the Cur-PLGA exhibited no cytotoxic effect on Normal mouse fibroblast cells (L-929) cell line. IC50 of Cur -PLGA increased 99.5% against Cur nanoparticles ($33.57 \pm 0.62 \mu\text{M}$) ($P < 0.05$).

Conclusion: In this study, we constructed a novel preparation of curcumin nanoparticles with PLGA and different grades of PVA (polyvinyl alcohol) and lecithin to improve the bioavailability of CUR and PLGA exhibited no cytotoxic effect on L-929 cell line

References: In this study, we constructed a novel preparation of curcumin nanoparticles with PLGA and different grades of PVA (polyvinyl alcohol) and lecithin to improve the bioavailability of CUR and PLGA exhibited no cytotoxic effect on L-929 cell line

Disclosure of Interests: None declared

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12. Rheumatoid arthritis - prognosis, predictors and outcome

AB0176 RISK OF ANTI-CITRULLINATED PEPTIDE ANTIBODIES AND RHEUMATOID FACTOR IN MALE SMOKERS: DATA FROM KUWAIT REGISTRY FOR RHEUMATIC DISEASES (KRRD)

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Background: Smoking has been proposed to be associated with the development of anti-citrullinated peptide antibodies (ACPA) in rheumatoid arthritis (RA) patients.

Objectives: To study the relationship between smoking and ACPA as well as smoking and RF in patients with RA in Kuwait Registry for Rheumatic Diseases (KRRD).

Methods: Data on RA patients were extracted from KRRD from four major hospitals from February 2013 through December 2019. As females rarely smoke in Kuwait with a smoking prevalence of 3% in female RA patients in KRRD, females were excluded from the study population to reach the minimum statistical percentage needed to perform chi square test and assess the association between smoking and other variables. Statistical tests were applied where appropriate. Logistic regression was conducted to adjust for possible confounders including