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Suuliste ettekannete teesid

Doktorantide sessiooni teesid

Paracrine factors from fibroblast aggregates in a fibrin matrix carrier enhance keratinocyte viability and migration

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Efficient reepithelialisation of skin lesions is dependent on paracrine support from connective tissue fibroblasts. We previously showed that mesenchymal cell activation evoked by direct cell-cell contacts, nemosis, produces massive amounts of HGF (hepatocyte growth factor) and that stimulation of keratinocyte migration by nemosis-derived factors utilizes the HGF/c-Met/PI3K pathway. In this study we aimed to show that, mimicking a dermal component, an active matrix incorporating factors released from nemosis-programmed fibroblasts supports keratinocyte migration and proliferation.

Multicellular spheroids were formed on U-bottomed 96-well plates treated with low-electroendosmotic agarose. Conditioned medium from spheroid cultures was combined with fibrin to establish a controlled-release gel. Cell respiration, an indicator of cell viability, was assayed by the mitochondria-dependent reduction of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) to formazan after seeding of primary keratinocytes on the fibrin matrix. Proliferation was analyzed by measuring fluorescence signal from gfp (green fluorescent protein) -labelled HaCaT cells.

The matrix supported viability and adherence of both primary keratinocytes and gfp-HaCaT cells, as evaluated by MTT assay and persistence of gfp-fluorescence. The fibrin-nemosis matrix promoted migration of keratinocytes to cover a larger area and this effect was inhibited by an EGFR/c-Met receptor tyrosine kinase inhibitor.

Pika ahelaga hüdroksü-atsüül-CoA dehüdrogenaasi defitsiidi esinemine Eestis

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Pika ahelaga hüdroksü-atsüül-CoA dehüdrogenaasi (*long-chain 3-hydroxyacyl-CoA dehydrogenase*, LCHAD) defitsiit on pärilik mitokondriaalne rasvhapete oksüdatsiooni (FAO) defekt. Haigus haarab erinevaid elundisüsteeme, kuid eeskätt kahjustuvad energiast sõltuvad koed. Klassikaliseks avaldumisvormiks on metaboolse stressi tingimustes kujunev hüpoglükeemia ja/või Reye' sündroom. Diagnoosimata ja ravimata haigusjuhtudel on laste suremus imiku- ja väikelapseeas suur (kuni 40%). Töö eesmärgiks on kasutusele võtta LCHAD puudulikkuse diagnostika atsüülkarnitiinide tandem mass-spektromeetrilise (MS/MS) analüüsi abil ning välja selgitada haiguse esinemissagedus Eestis.

Töö esimeses osas analüüsiti retrospektiivselt 1000 vastsündinut ja 50 sümptomaatilise rasvhapete oksüdatsiooni defektide kahtlusega patsienti HADHA geenis asuva sagedasema mutatsiooni c.1528G>C kandluse suhtes (87%-l mutantsetest alleelidest). Töö teises osas tehti prospektiivne FAO defektide selektiivne skriining sümptomaatilistele patsientidele (250 patsienti). Selleks kasutati kehavedelike atsüülkarnitiinide tandem MS/MS analüüsi, kuna LCHAD defitsiidi korral akumuleeruvad mitokondri maatriksis pika ahelaga atsüül-CoA-d, mis transporditakse rakust välja atsüülkarnitiinidena.

Mutatsiooni c.1528G>C kandlus Eesti vastsündinute hulgas on 1 : 200, mis on sarnane meie naaberriikidega (Soome 1 : 240, Poola 1 : 216) ja sagedasem kui Lääne-Euroopas (Holland 1 : 680). Retrospektiivse ja prospektiivse valik-skriiningu tulemusena on diagnoositud LCHAD puudulikkust 4 lapsel 3 perekonnast. Kahel juhul on diagnoos ja ravi alustamine olnud õigeaegne. LCHAD puudulikkus on sagedasim FAO defekt Eestis, mistõttu peaks tulevikus kaaluma selle haiguse suhtes testimise lisamist vastsündinute mass-skriiningprogrammi.

PSORS1C1, PSORS1C2 geenidekspressioon psoriaasi korral

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Psoriaas on mitmeteguriline krooniline põletikuline nahahaigus, mille patogeneesis on leitud oluline roll T-rakkudel, APCdel, keratinotsüütidel, Langerhansi rakkudel, makrofaagidel, NK-rakkudel, mitmetel kasvufaktoritel ning tsütokiinidel. Varem on uuritud PSORS1 (*psoriasis susceptibility gene* 1) kui ühe olulisema psoriaasi predisponeeriva faktori geneetilist tausta haiguse tekkel (Carlen jt, 2007), kuid pole tehtud PSORS1C1 ja PSORS1C2 ekspressiooniuuringuid.

Töö eesmärgiks oli näidata PSORS1, PSORS1C1 ja PSORS1C2 ekspressiooni psoriaasihaigete kahjustatud ja kahjustamata nahas võrrelduna kontrollrühmaga. Lisaks näitasime geenide rakuliini spetsiifilist

lokalisatsiooni primaarkultuuri keratinotsüütides, melanotsüütides ja fibroblastides. Nahakoe proovid koguti TÜ Kliinikumi nahakliinikusse ja Tallinna Lastehaiglasse pöördunud psoriaasihaigetelt ning kontrollrühma patsientidelt. RNA eraldasime nii haigete kahjustatud kui ka kahjustumata nahast, kontrollrühma koeproovist ning kontrollnahast kultiveeritud rakukultuuridest. Geenikspressiooni määramiseks kasutasime QRT-PCR meetodit.

Psoriaasihaige terves nahas oli PSORS1C1 ekspressioon tunduvalt suurenenud võrreldes psoriaasihaige haige nahaga ning kontrollrühmaga ($p < 0,01$). Sama tendents oli näha ka PSORS1 korral. Kontrollrühma rakuliinide fibroblastides ilmnes PSORS1C1 kõrgem ekspressioon kui sama nahakoe proovi melanotsüütides.

PSORS1C2 oli enam ekspresseeritud psoriaasihaige haiges ($p < 0,0009$) ja terves ($p < 0,05$) nahas võrrelduna kontrollrühmaga. PSORS1C2 ekspresseerub nimetatud kolmest rakutüübist vaid keratinotsüütides. Uuringu tulemused näitavad PSORS1 lookuse olulisust psoriaasi patogeneesis ning kirjeldavad haiguses osalevate geenide rakuspetsiifilist ekspressiooni.

CCR5 haplotüübipaarid mõjutavad HIV-1 ja HCV infektsiooni nakatumist süstivate narkomaanide populatsioonis

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EESMÄRK. Hinnata CCR5 haplotüübipaaride ning HIV-1 ja HCV positiivsuse vahelisi seoseid Eesti süstivate narkomaanide (SN) populatsioonis.

MEETODID. Uuringusse kaasati 374 SNi kahest süstlavahetusprogrammist ja kolmest Eesti vanglast aastatel 2006–2007. Populatsiooni keskmine vanus oli 26 a, enamus mehed (85%) ja 95% antiretroviirusliku ravi naiivsed. 56% uuritavatest olid HIV-positiivsed (HIV+), 76% HCV-positiivsed (HCV+) ja 47% HIV/HCV koinfektsiooniga. CCR5 polümorfismid määrati genoomselt DNA-lt reaalaja PCRiga ning haplotüübipaaride defineerimisel kasutati CCR5 polümorfismide evolutsioonilist klassifikatsiooni. Varem oli samal populatsioonil määratud CCL3L1 koopiaarv.

TULEMUSED. Kõige sagedasemad CCR5 haplotüübid olid HHE (*HH-human haplotype*) (32%), HHC (29%) ja HHG2 (12%) ning haplotüübipaaridest HHC/HHE (18%), HHE/HHE (12%) ja HHC/HHC (10%). HHF2/HHE jaotuvus erines oluliselt HIV+ ja HIV– rühmas (4% *vs* 10%; $p < 0,05$) ja HHG1/HHE HCV+ ja HCV– grupis (3% *vs* 19%; $p < 0,05$). Multivariantne logistiline regressioonanalüüs, mis oli kohandatud soole, koinfektsiooni esinemisele, CCL3L1 koopiaarvule ja süstitavate narkootikumide kasutusajale, näitas, et isikutel, kel oli HHF2/HHE, olid väiksemad šansid olla HIV+ (OR 0,16; 95% CI 0,05–0,52) kui isikutel, kel oli mõni teine haplotüübipaar. Kasutades identset mudelit, leiti, et HHG1/HHE omamine vähendas šanssi olla HCV+ (OR 0,02; 95% CI 0,00–0,16) võrreldes isikutega, kel seda haplotüübipaari ei olnud.

JÄRELDUS. CCR5 haplotüübipaaridel võib olla oluline osa nii HIVsse kui ka HCVsse nakatumisel.

Genotype-specific natural history of HPV infections among mothers in the Finnish Family HPV Study

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To understand the natural history of HPV-infections more information is needed on genotype-specific HPV-infections.

In the Finnish Family HPV Study, 329 pregnant women were recruited, and followed-up for 6 years. Multiplex-HPV-genotyping was used to define genotype-specific prevalence at each visit. Generalized estimating equation models and Poisson regression were constructed to estimate predictors of virus acquisition, clearance and persistence.

HPV16 was the most common genotype. HPV16 together with multiple-type infection were the most frequent incident infections and showed the lowest clearance frequency. The actuarial incidence and clearance rates were also highest for HPV16 and multiple-type infections. Altogether LR-HPV types showed lower incidence and clearance times than HR-HPV types. Persistence was most prolonged for HPV35 and 58. Independent protective factors against incident infections were higher number of life-time sexual partners, initiation of OC use after age 20 and becoming pregnant during FU. Older age and negative oral HR-HPV DNA status at baseline were associated with increased clearance, whereas higher number of current sexual partners decreased the probability of clearance. Persistence was increased with early onset of smoking, practicing oral sex and older age. HPV16 together with multiple-type infections were the most frequent in incidence, most likely to remain persistent and least likely to clear.

In multivariate models, different predictors were associated with these main viral outcomes, and there is some tentative evidence to suggest that oral mucosa might play a role in controlling these outcome events.

The effect of maternal thyroid dysfunction on pregnancy outcome, peri- and neonatal period and on the later health of mothers. Prospective population-based cohort study

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Normal maternal thyroid function is crucial for the development of the child. Maternal thyroid dysfunction can possibly lead to adverse pregnancy and perinatal outcome as well as compromise the later health of both mothers and children. However, information on this is scarce.

This study aims to find out if maternal thyroid dysfunction or antibodies have an effect on pregnancy complications, perinatal complications, and subsequent maternal risk to conduct thyroid diseases and to create gestational age specific reference intervals for thyroid hormones.

This is established using as a study population the prospective population-based Northern Finland Birth Cohort 1986 with extensive data from early pregnancy onwards. The cohort data has been collected with questionnaires and clinical trials and expanded using national databases. Maternal thyroid function has been evaluated by analyzing thyroid hormones and antibodies from early pregnancy serum samples.

The results of this study showed that maternal hypothyroidism is not associated with any serious pregnancy or perinatal outcome, however, hyperthyroidism increased the risk of having a large infant. Thyroid antibody-positivity increased the risk of perinatal death. Maternal hypothyroidism and thyroid antibodies were associated with increased risk for later thyroid diseases.

We conclude that especially maternal thyroid antibodies may be harmful for the child during pregnancy and detection of antibody-positive mothers is advisable. Thyroid dysfunction and antibodies during pregnancy predicted subsequent thyroid diseases, and identifying high-risk women may prove useful in preventing and detecting these diseases.

Familial longevity is marked by enhanced insulin sensitivity

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A key question in aging research is whether the extreme effects of altered insulin signalling on lifespan observed in model organisms can be translated to humans. Our aim was to compare tissue specific insulin action between subjects from long-lived families and controls.

We performed a two step hyperinsulinemic-euglycemic clamp in 12 offspring from long-lived siblings and 12 of their partners, aged 52–72 years. All were healthy, non-smoking and non-obese.

The groups were similar with regard to sex distribution, age, exercise, BMI, waist circumference and fat mass. Glucose infusion rates required to maintain euglycemia during high dose insulin infusion were significantly higher ($p = 0.036$) in offspring from long-lived siblings, reflecting higher whole body insulin sensitivity. Insulin-mediated glucose disposal rate was higher in offspring than in controls (42.5 ± 2.7 *vs* 33.2 ± 2.7 μmol/kg x min, mean ± SE, $p = 0.025$). The capacity of insulin to suppress endogenous glucose production and lipolysis did not differ between groups (all $p > 0.05$). Furthermore, glucose disposal rate was significantly correlated with the mean age of death of the parents.

In conclusion, subjects from long-lived families are marked by enhanced insulin sensitivity and mimic the phenotype found in mammalian models with genetic disruption of IGF-1/insulin signal transduction. These observations allow to identify biomolecular mechanisms to promote health in old age.

Sedentary lifestyle and emergence of hopelessness in middle-aged men

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A sedentary lifestyle and psychosocial factors such as hopelessness and depression increase cardiovascular risk. Cross-sectional evidence suggests positive effects of physical exercise on psychological well-being, but the time order of the relationship between physical activity and hopelessness has not been addressed.

DESIGN. Population-based prospective cohort study with 630 middle-aged men participating in the 4-year follow-up and 509 men in the 11-year follow-up.

METHODS. We investigated the association of leisure-time physical activity (LTPA) with the development of hopelessness during the follow-up. LTPA and hopelessness were quantified with questionnaires.

RESULTS. In cross-sectional analyses, LTPA was inversely associated with hopelessness, independently of depression. Among men who did not have feelings of hopelessness at baseline, those who reported engaging in at least 2.5 h/wk of moderate-to-vigorous physical activity had a lower risk (OR 0.65, 95% Cl 0.39-1.09, P for the trend = 0.047) to feel hopeless than sedentary men 4 years later after adjustment for age, smoking, alcohol consumption, cardiovascular disease and socioeconomic status. Furthermore, this relationship was not explained by body mass index or maximal oxygen uptake. Adjustment for depressive symptoms slightly weakened the association (OR 0.66, 95% Cl 0.39-1.11, P for the trend = 0.060). After 11 years, men who reported being physically active at baseline were still less likely to feel hopeless, after adjusting for age. However, after further adjustment for other confounding factors the association was no longer significant.

CONCLUSIONS. Moderate-to-vigorous physical activity seems to prevent development of hopelessness in middle-aged men. This protective effect may diminish over time.

Predictive value of the Belgian outcome in burn injury prediction model

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Severe burn injuries are still responsible for a considerable morbidity and mortality, and therefore risk assessment is pivotal in research, resource allocation and clinical decision making. Our aim was to develop and apply a practical mortality prediction model for patients with severe burn injury.

The Belgian Outcome in Burn Injury (BOBI) prediction model was developed on basis of a multi-centre Belgian cohort (1999–2003, n = 5246), and consists out of a 0-10 point score based on 3 major predictors for mortality: increasing age and total burned surface area, and the presence of inhalation injury. Its accuracy was determined with a Belgian (2004, n = 981) and Hungarian cohort (1999–2005, n = 2326), and with the population admitted at the Ghent burn unit (Belgium, 1985–2004, n = 1385),

incl. 2 subpopulations with bloodstream infections - BSI (n = 76, 1992–2006) and ventilator associated pneumonia - VAP (n = 46, 2002–2010).

ROC-curve analysis showed areas under the curve (AUC) between 0.94-0.95 for the Belgian and the Hungarian cohort, and 0.88 and 0.79 for the subpopulations with BSI and VAP respectively. These AUC's imply a good sensitivity and specificity. The predicted mortality was higher than observed in the BSI and VAP subgroups, which can be due to survival bias. Moreover, the BOBI-model is designed to estimate the risk of death on admission, and does not take into account subsequent events complicating the course of the patient.

To conclude, we developed an easy-to-use prediction model, which proved to be accurate in distinct populations with severe burn injury.

Kas 6kuuline sõjaline missioon Afganistanis mõjutab põletikumarkerite ja D-vitamiini taset ning arteriaalset jäikust?

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Ülemääraselt raske koormus võib avaldada pöörduvat kahjulikku toimet südame vasaku vatsakese funktsioonile ja arteriaalsele jäikusele. Lisaks on näidatud, et raske kestev vastupidavustreening kutsub lühiajaliselt esile süsteemset põletikku. Viimasel ajal on leitud, et D-vitamiin on potentsiaalselt põletikuvastase toimega.

Käesoleva uuringu eesmärgiks oli selgitada 6-kuulise sõjalise missiooni mõju arteriaalsele jäikusele, põletikumarkerite ja D-vitamiini tasemele. 65 sõdurile (keskmine vanus 26 ± 4 aastat) teostati uuringud enne ja pärast 6kuulist sõjalist missiooni Afganistanis. Arteriaalset jäikust mõõdeti pulsilaine leviku kiiruse aordis registreerimise ja pulsilaine analüüsi abil. Põletikumarkerid määrati verest ELISA-meetodil, seerumi 25-hüdroksüvitamiin D taset mõõdeti radioimmuunmeetodil. Arteriaalne jäikus, perifeerne ja tsentraalne vererõhk ei muutunud statistiliselt missiooni käigus. D-vitamiini tase tõusis 2,6 korda (40 ± 15 *vs* 104 ± 24 (nmol/l), $p < 0,001$). Oluliselt tõusis ka kõrgtundliku C-reaktiivse valgu tase (0,68 ± 0,7 *vs* 1,47 ± 3,55 (mg/l), $p = 0,03$), leukotsüütide arv (5,4 ± 1,1 *vs* 6,3 ± 1 (x109/l), $p < 0,001$) ning mitmete proinflammatoorsete tsütokiinide tase, sealhulgas IL-1α (0,13 ± 0,18 *vs* 0,2 ± 0,23 (pg/ml), $p < 0,001$), IFN-γ (2,6 ± 2,4 *vs* 5,3 ± 3,4 (pg/ml), $p < 0,001$) ja MCP-1 (151 ± 61 *vs* 229 ± 95 (pg/ml), $p < 0,001$).

Sõjalise missiooni kurnavates tingimustes jäi arteriaalne jäikus muutumatuks. Samas esines olulisi muutusi põletikumarkerite spektris ja D-vitamiini tasemes. Me oletame, et oluliselt tõusnud D-vitamiini tase võis vähendada võimalikke põletikust tingitud muutusi arteriaalses jäikuses.