kines, TNFα or IL-1β, on early osteogenesis in vitro. Under osteogenic conditions, IL-1β was found to inhibit cell proliferation in a dose dependent manner, whereas TNFα exhibited no effect. Histochemical examination revealed the presence of either TNFα or IL-1β to dramatically decreased mineralization in a dose dependent manner. Q-PCR analysis indicated that in the presence of IL-1β, despite increased expression of bone-specific alkaline phosphatase (Akp2) mRNA, levels of other osteogenesis markers (Runx2, Col1a and Sp7) were decreased. In the presence of TNFα, levels of Akp2, Runx2 and Sp7 were all decreased. Our findings indicate that the influence of early mesenchymal progenitor cells on bone remodelling may be substantially altered in the presence of proinflammatory cytokines.

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FC08.6

NF-κB P65 SUB-UNIT REPRESSES ANTIOXIDANT RESPONSIVE ELEMENT (ARE) -DEPENDENT GENE EXPRESSION BY DEPRIVATION OF CBP FROM NRF2 AND RECRUITING HISTONE DEACETYLASES 3 TO ARE THROUGH FORMING COMPLEX WITH CBP-MAFK

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Using ARE-driven and NF-κB-targeted reporter genes, transfection of the NF-κB p65 subunit and Nrf2 into HepG2 or other cells, as well as siRNA technique to knockdown endogenous p65 in cells, we found that NF-κB p65 subunit repressed the anti-inflammatory and anti-carcinogenetic Nrf2-ARE pathway at transcriptional level. In p65-overexpressed cells, the ARE-dependent expression of heme oxygenase-1 was strongly repressed. In the cells where NF-κB and Nrf2 were simultaneously activated, p65 unidirectionally antagonized Nrf2 transcriptional activity. The p65-mediated ARE inhibition was independent of the transcriptional and DNA-binding activities of p65. Co-transfection and RNA interference experiments revealed two mechanisms which coordinate the p65-mediated repression of ARE: (1) p65 selectively deprives CREB binding protein (CBP) from Nrf2, but not MafK, by competitive interaction with the CH1-KIX domain of CBP, resulting in inactivation of Nrf2 transactivation domain and concomitant abrogation of the Nrf2-stimulated coactivator activity of CBP; (2) p65 promotes recruitment of histone deacetylases 3 (HDAC3) to ARE by enhancing the interaction of HDAC3 with either CBP or MafK, leading to inactivation of CBP and deacetylation of MafK. This study may establish a novel pro-inflammatory and pro-carcinogenic model for the transrepression of the ARE-dependent gene expression by p65 subunit. Since various inflammatory and tumor tissues constitutively overexpresses p65 in their nuclei, the finding in this study implies a strong repression of ARE-dependent gene expression must take place in those tissues. In this regard, the findings in this study may help to explain why oxidative stresses and toxic insults usually occur in those pathological loci.

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FC08.7

THROMBIN INDUCES CHEMOTAXIS OF MATURED DENDRITIC CELLS VIA RHO/ERK PATHWAYS

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Dendritic cells (DC) play a pivotal role in the induction of immune response and tolerance. It is less known that DC accumulate in atherosclerotic arteries, where they might activate T-cells and contribute to the progression of disease. The serine protease thrombin is the main effector protease of the coagulation cascade. Thrombin is also generated at sites of vascular injury and during inflammation. Hence, thrombin generation is observed within atherosclerotic and other inflammatory lesions including rheumatoid arthitis. Thrombin activates various cells via protease-activated receptors (PARs). Immature DC do not express PARs. Upon maturation with LPS, TNF-αlpha, or CD40L, only LPS-matured DC expressed PAR1 and PAR3 on their surface. Stimulation of DC with thrombin, PAR1- or PAR3-activating peptides elicited actin polymerization and concentration-dependent chemotactic responses in LPS-, but not in TNF-αlpha-matured DC. The thrombin-induced migration was a true chemotaxis as assessed by checkerboard analysis. Stimulation of PARs with thrombin or respective receptor-activating peptides led to activation of ERK1/2 and Rho kinase I (ROCK-I) as well as subsequent phosphorylation of the regulatory myosin light chain 2 (MLC2). The ERK1/2- and ROCK-I-mediated phosphorylation of MLC2 was indispensable for the PAR-mediated chemotaxis as shown by use of pharmacological inhibitors of ROCK, ERK and MLC kinases. In addition, thrombin significantly increased the ability of mature DC to activate proliferation of naive T-lymphocytes in mixed leukocyte reactions. In conclusion, our work demonstrates expression of functionally active thrombin receptors on LPS-matured DC. We identified thrombin as a potent chemoattractant for mature DC, acting via Rho/ERK-signaling pathways.

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