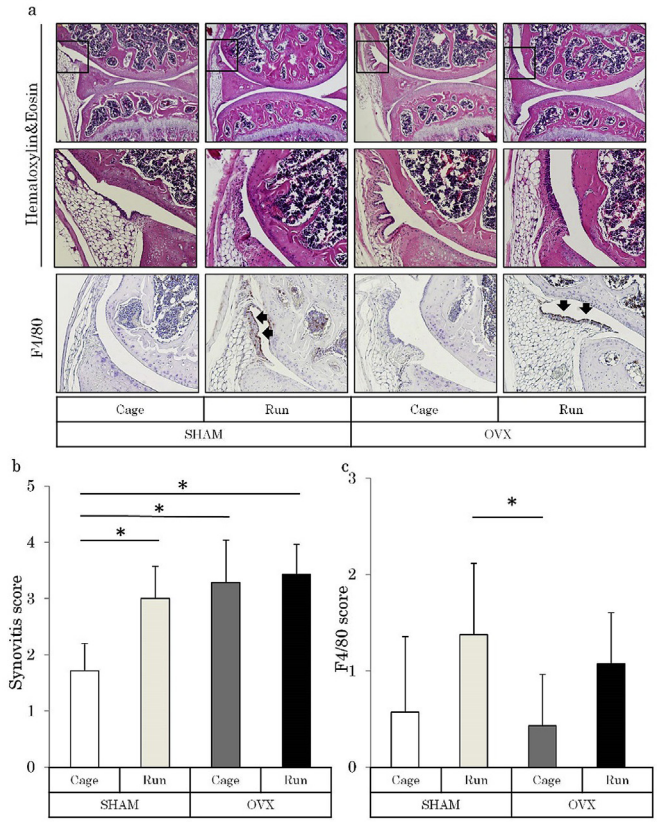


Fig. 2.

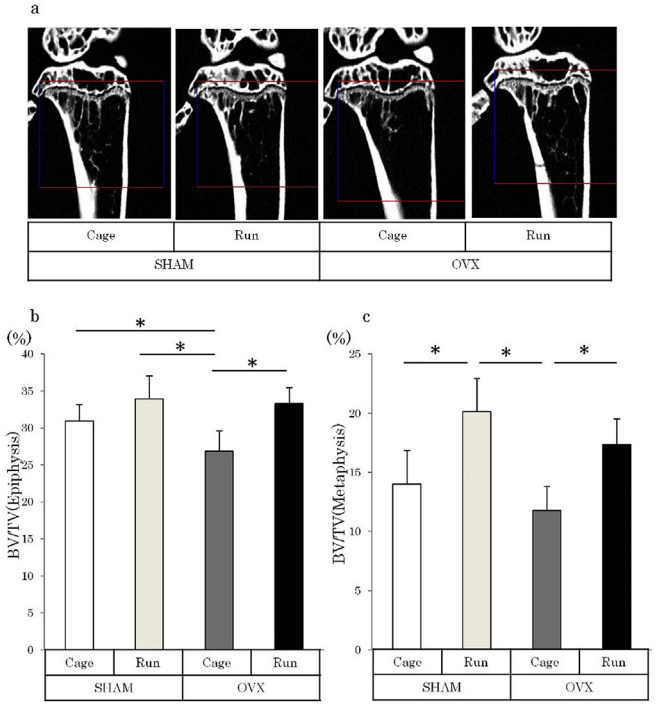


Fig. 3.

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## INVOLVEMENT OF TGF-A, ROCK1, DOCK2 AND CAVEOLIN-1 IN OSTEOARTHRITIS PATHOGENESIS

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**Purpose**: Osteoarthritis (OA) is a debilitating disease of the joints characterized by cartilage degradation. To date there is no available pharmacological treatment to inhibit disease progression, neither is there a suitable biomarker for early disease prognosis. The aim of the present study was to investigate the molecular pathways involved between four identified OA susceptibility genes and their role in OA pathogenesis.
**Methods**: We initially performed a Genome-Wide Association Study (GWAS) in DNA samples isolated from OA patients that underwent knee replacement surgery and healthy individuals with no history of joint disease, all of Greek descent. Analysis of GWAS results revealed significant association between single nucleotide polymorphisms (SNPs) in transforming growth factor alpha (TGF-α), caveolin-1, rho-associated coiled coil containing protein kinase 1 (ROCK1) and dedicator of cytokinesis-2 (DOCK2) genes and OA. We next proceeded with evaluation of mRNA and protein expression levels of TGF-α, ROCK1, DOCK2 and caveolin-1 by real time PCR and western blotting. Normal articular chondrocytes were next treated with TGF-α and its effect was assessed by mRNA and protein expression analysis, to identify the genes/proteins affected and the signaling cascade activated.
**Results**: Our results showed that OA chondrocytes demonstrate significantly higher TGF-α expression levels compared to normal chondrocytes, while the inverse was observed for the expression of ROCK1, caveolin-1 and DOCK2. Treatment of normal chondrocytes with TGF-α resulted in activation of ROCK-1 (Rho kinase-1), caveolin-1 and dedicator of cytokinesis-2 (DOCK2) with concurrent upregulation of catabolic factors, such as matrix metalloproteinase-13 (MMP-13). The signaling cascade involved was also investigated.
**Conclusions**: The suggestion by previous studies that TGF-α and ROCK1 are involved in OA pathogenesis was verified in the present study by GWAS and expression analysis, while two novel genes, caveolin-1 and DOCK2 are postulated for the first time to be implicated in disease mechanism and in response to TGF- α.

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## THE ROLE OF NRF2 TRANSCRIPTION FACTOR IN OSTEOARTHRITIS

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**Purpose**: Osteoarthritis (OA) is the most common joint disorder and a leading cause of physical disability. Over the past years, we studied the complex role of the lipid peroxidation product 4-hydroxynonenal (HNE) in osteoarthritis (OA). We are the first to demonstrate that HNE level was higher in patients with OA as compared to healthy subjects. Moreover, we demonstrated that HNE induces a cascade of catabolic and inflammatory events involved in OA process. We recently showed that the expression of glutathione-s-transferase A4-4 (*GSTA4-4*), a gene encoding the HNE-conjugating enzyme GSTA4-4, as well as nuclear factor erythroid 2-related factor 2 (Nrf2), which regulates *GSTA4-4* gene expression, is decreased in human OA cartilage compared to controls. The objective of the present study is to explore the effects of protandim, activator of Nrf2, on GSTA4-4 regulation, IL-1β-induced catabolic and inflammatory responses and $H_2O_2$-induced oxidative stress.
**Methods**: Human OA chondrocytes were pre-treated with different concentrations of protandim for 1 hour followed by treatment with IL-1β or H2O2 for 24 hours. Metalloproteinase-13 (MMP-13), nitric oxide (NO), prostaglandin E2 (PGE2) and HNE were determined using commercial kits. GSTA4-4 mRNA level was assessed by real-time PCR
**Results**: Our findings showed that protandim abolished IL-1β-induced MMP-13, NO, and $PGE_2$ production as well as H2O2-induced HNE generation. The effect of protandim is mediated, in part, by GSTA4-4 up-regulation
**Conclusions**: Collectively, these data strongly represent a new mechanism for the control of Nrf2 and GSTA4-4 expression in OA. Indeed, targeting mechanisms underlying their expression could be a promising avenue in OA treatment.