Cyclin-dependent kinase inhibitor-1 deficiency increases susceptibility to osteoarthritis with inflammation

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Abstract introduction: Cyclin-dependent kinases (CDKs) are recognized as a regulator of cell cycle progression. p21 is reported as one of CDK inhibitors (CKIs) which regulates activation of CDKs. Recently Marves reported that p21 deficiency mice was susceptible to LPS-induced inflammation. However, how p21 works responsible for persistent inflammation of joints that occurs with osteoarthritis is unknown. Therefore, the aim of this study is to investigate the p21 function about progressions of osteoarthritis in vitro and vivo.

Methods: p21 knockout mice (B6.129S6(Cg)-Cdkn1a1tm1Led/J) and C57Bl/6J (Wild type; Wt) mice were obtained from Jackson Laboratory. For vitro study, chondrocytes were isolated respectively from the knee joint of mouse at age of day 3. Monolayer culture were incubated in 30 mm² dishes at 3×10⁶ chondrocyte in 2ml DMEM containing FBS and penicillin/streptomycin overnight. It was divided into three groups as following: non-treatment control, treatment with 10ng/ml IL-1β for 24 hours, and treatment without IL-1β for 24 hours. In vivo study, respectively 84 male mice at 10 week-old were used. We operated Destabilization of the medial meniscus (DMM) surgery to the right knee and did sham surgery to the left knee. We sacrificed and collected knee joints and blood samples at 7 time points, non-operative group (control), postoperative 1day, 3day, 1week, 2weeks, 4weeks, and 8weeks. Each group contained 6 male mice. Morphometric bone change of knee joint was evaluated by micro-CT. Sections were stained with H-E, Safranin O, and expression of F4/80, IL-1β, p-IKK α/β, MMP-13 were analyzed by immunohistochemistry.

Result section:

Micro-CT analysis: p21-/- mice showed joint destruction with ectopic ossification as compared with Wt mice (Figure 1a). Tibia-epiphysis analysis showed significantly higher BS/TV, trabecular separation and decreased subchondral bone thickness, trabecular thickness in p21-/- mice (Fig. 1c,d,e,f). However, BV/TV and BMD did not indicate significant difference (Fig. 1b,g). Histological evaluation: Safranin O staining in joint cartilage tissues were evaluated by OARSI histopathology classification. p21-/- mice post DMM surgery 8 weeks showed irregular articular surface, superficial clefs and proteoglycan content loss. OARSI histopathology classification score in p21-/- mice was significantly higher as compared with Wt mice (Fig. 2). We evaluated severity of synovitis with OARSI recommended scoring system in H-E stain section. p21-/- mice showed outgrowth of synovial membrane and evaluated higher severity score in both 1 and 8 weeks (Fig. 3). Immunohistochemical analysis showed that F4/80, IL-1β, p-IKK α/β and MMP-13 were more expressed in both synovium and cartilage of p21-/- mice (Fig. 4). ELISA: Serum levels of IL-1β were significantly elevated in p21-/- mice at each time points except control group (Fig. 5). Real time RT-PCR analysis: IL-1β increased the expressions levels of MMP-13 and MMP-3 mRNA in both WT and p21-/- mice group. When we compared the MMPs expressions, both MMP-3 and 13 expression levels were significantly elevated in p21-/- mice (Fig. 6a,b).

Discussion: Previous study reported that p21 reduce the secretion of IL-1β by p21 knockout macrophages in vitro study. We demonstrated that p21 deficiency increased the expression of IL-1β systemically in OA progression, and increased inflammation markers and catabolic factors such as MMPs in joint. Further, we confirmed that chondrocytes isolated from p21-/- mice were more susceptible to increase the expressions of IL-1β-induced MMPs. Therefore, we concluded that p21 deficiency is susceptible to osteoarthritis with inflammation.

Significance: p21 deficiency was susceptible to osteoarthritis change with increase of inflammation markers expression in both systemically and locally.


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