INTISARI
PENGARUH KNOCK OUT GEN *BMPR2* TERHADAP FUNGSI MAKROFAG RAW264.7 PADA PENSINYALAN SELULER DAN INTEGRITAS PEMBULUH DARAH

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Latar Belakang: Gen *BMPR2* berperan dalam menjaga homeostasis fungsi sistem imun. Makrofag merupakan komponen sistem imun yang regulasinya diatur oleh *BMPR2*. Mutasi *BMPR2* pada makrofag tidak hanya mengganggu fungsi, tetapi juga mengganggu berbagai pensinyalan selulernya yang dapat diamati melalui ekspresi CD68, KCNA2, dan CLIC1. Interaksi makrofag disfungsional dengan pembuluh darah dapat mengganggu integritas vaskuler.
Tujuan: mengkaji pengaruh *knock out* (KO) gen *BMPR2* terhadap ekspresi mRNA CD68, KCNA2, dan CLIC1 pada makrofag secara *in-vitro* dan mengkaji perubahan integritas pembuluh darah serta ekspresi mRNA CD68, KCNA2, CLIC1, dan GLUT4 secara *ex-vivo* sebagai respon terhadap gangguan pensinyalan seluler akibat paparan supernatan makrofag *BMPR2* KO
Metode: C*ell line* makrofag RAW 264.7 dilakukan *knock out* gen *BMPR2* menggunakan metode CRISPR/Cas9 (Kelompok *BMPR2* KO, n=3), kemudian ekspresi mRNA CD68, KCNA2, dan CLIC1 dibandingkan dengan makrofag *BMPR2 wildtype (WT) (n=3)*. Selanjutnya, enam aorta yang diekstraksi dari mencit jantan usia 3 bulan dibagi menjadi 2 kelompok yang dikultur dalam *conditioned medium* berisikan supernatan makrofag *BMPR2* KO (n=3) dan *BMPR2* WT (n=3) dengan konsentrasi 75% supernatan dan 25% DMEM. Setelah 48 jam, gambaran histologis aorta dengan pewarnaan HE dan pengukuran ekspresi mRNA CD68, KCNA2, CLIC1, dan GLUT4 diamati. Pengukuran ekspresi mRNA makrofag *in-vitro* dan aorta *ex-vivo* dilakukan dengan RT-qPCR.
Hasil: Ekspresi mRNA CD68 dan KCNA2 lebih rendah serta CLIC1 lebih tinggi signifikan ($p<0.05$) pada makrofag *BMPR2* KO dibandingkan *BMPR2* WT. Paparan supernatan makrofag *BMPR2* KO pada aorta menyebabkan gangguan integritas vaskuler secara histopatologis dan ekspresi mRNA CD68, KCNA2, CLIC1, dan GLUT4 yang lebih tinggi ($p<0.05$) dibandingkan kelompok kontrol.
Kesimpulan: *Knock out BMPR2* menyebabkan disfungsi makrofag melalui perubahan ekspresi CD68, KCNA2, dan CLIC1. Akibatnya, pensinyalan seluler pada pembuluh darah mengalami perubahan dan integritas vaskuler terganggu.
Kata Kunci: *BMPR2*, makrofag, pro-inflamasi, pro-proliferatif, aorta, CD68, KCNA2, CLIC1, GLUT4

ABSTRACT
EFFECT OF *BMPR2* GENE KNOCK OUT ON RAW264.7 MACROPHAGE FUNCTION ON CELLULAR SIGNALING AND VASCULAR INTEGRITY

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Introduction: *BMPR2* plays important role in maintaining homeostasis of immune system function. Macrophages are components of the immune system whose regulation is established by *BMPR2*. *BMPR2* mutation in macrophages not only impair function, but also impair their various cellular signaling which can be observed through the expression of CD68, KCNA2, and CLIC1. The interaction of dysfunctional macrophages with blood vessels can impair vascular integrity.
Aim: To study the effect of *BMPR2* gene knockout on CD68, KCNA2, and CLIC1 mRNA expression in macrophages *in-vitro* and investigate changes in blood vessel integrity and *ex-vivo* expression of CD68, KCNA2, CLIC1, and GLUT4 mRNA in response to cellular signaling disturbances due to exposure to macrophage *BMPR2* KO supernatant
Methods: RAW 264.7 macrophage cell line was knocked out of the *BMPR2* gene using the CRISPR/Cas9 method (*BMPR2* KO group, n=3), then mRNA expression of CD68, KCNA2, and CLIC1 was compared with wildtype (WT) *BMPR2* macrophages (n=3). Furthermore, six aortas extracted from 3-months-old male mice were divided into 2 groups which were cultured in conditioned medium containing macrophages *BMPR2* KO (n=3) and *BMPR2* WT (n=3) supernatants with concentration of 75% supernatant and 25% DMEM each. After 48 h, aorta histology slide with HE staining and measurement of CD68, KCNA2, CLIC1, and GLUT4 mRNA expression were observed. mRNA expression of macrophage *in-vitro* and aorta *ex-vivo* was performed using RT-qPCR.
Results: CD68 and KCNA2 mRNA expression was lower while CLIC1 was higher significantly ($p<0.05$) in *BMPR2* KO macrophages compared to *BMPR2* WT. Exposure to *BMPR2* KO macrophages supernatant showed impaired vascular integrity and higher mRNA expression of CD68, KCNA2, CLIC1, and GLUT4 in the aorta ($p<0.05$) compared to control group.
Conclusion: *BMPR2* knockout causes macrophage dysfunction through altered expression of CD68, KCNA2, and CLIC1. As the result, cellular signaling and vascular integrity of blood vessels are compromised.
Keywords: *BMPR2*, pro-inflammatory, pro-proliferative, aortic, CD68, KCNA2, CLIC1, GLUT4