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Pengaruh Sekretom Fibroblas dari Adenokarsinoma Kolorektal terhadap Sifat Kepuncaan Sel Lestari HT-29: Tinjauan pada Ekspresi STAT3, ALDH1A1, OCT4, dan MnSOD = The Effect of Fibroblast Secretome from Colorectal Adenocarcinoma on The Stemness of HT-29 Cell Line: Focus on STAT3, ALDH1A1, OCT4 and Mnsod Expression

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Abstrak

Tingginya rekurensi pada kanker kolorektal (KKR) disebabkan karena terapi saat ini belum mempertimbangkan keberadaan lingkungan mikrotumor dan kepuncaan. Kepuncaan adalah sifat dari sel punca kanker yang memiliki kemampuan self-renewal, pluripotensi, dan tumorigenic. Penelitian kami sebelumnya menunjukan bahwa sekretom fibroblas dari adenokarsinoma kolorektal meningkatkan ekspresi CD133 dan CD44 dalam sel Lestari HT-29. Namun peran sekretom fibroblas tersebut terhadap sifat kepuncaan masih perlu diteliti lebih lanjut. Penelitian ini bertujuan untuk mengetahui pengaruh sekretom fibroblas adenokarsinoma kolorektal area tumor dibandingkan area nontumor dan fibroblas normal terhadap sifat kepuncaan, MnSOD, dan STAT3 pada sel lestari HT-29. Pemberian Conditioned Medium (CM) yang mengandung sekretom fibroblas adenokarsinoma kolorektal area tumor (CM-T) dan nontumor pasangannya (CM-NT) pada kultur sel lestari HT-29 sudah dilakukan oleh peneliti sebelumnya. Fibroblas normal (NF) diisolasi dari jaringan preputium. Semua fibroblas ditanam dalam media kultur bebas serum selama 24 jam untuk mengumpulkan CM. Kemudian, CM ditambahkan ke kultur sel lestari HT-29 selama 72 jam. Ekspresi mRNA Oct4 dan ALDH1A1 dianalisis dengan qRT-PCR. Ekspresi protein STAT3, pSTAT3, SOD, dan Oct4 dianalisis dengan western blot. Pemberian CM-T meningkatkan ekspresi mRNA OCT4 dan ALDH1A1 signifikan dibandingkan dengan kontrol, CM-NT, dan CM-NF. Kami menyimpulkan bahwa pemberian sekretom fibroblas KKR meningkatkan ekspresi OCT4 dan ALDH1A1 secara signifikan dibandingkan dengan fibroblas non tumor pasangannya dan fibroblas normal

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The high recurrence in colorectal cancer (CRC) is because current therapy has not considered the presence of a tumor microenvironment and stemness. Stemness is a characteristic of cancer stem cells that have properties like self-renewal, pluripotent and tumorigenic abilities. Our previous study has demonstrated that the secretomes of fibroblasts isolated from colorectal carcinoma (CRC) patients could upregulated the expression of CD133 and CD44 in the HT29 CRC cell line. However, the role of CRC fibroblasts secretomes in CRC stemness is needed to be further investigated. Therefore, the present study aimed to investigate the effect of fibroblast secretomes from CRC patients in comparison with the secretomes from normal fibroblasts on the expression of stemness markers, MnSOD, and STAT3 on HT-29 cells. The supplementation of Conditioned Medium (CM) from adenocarcinoma colorectal fibroblast (CM-T) and its nontumor partner (CM-NT) in HT-29 cell cultures has been done by our previous study. Normal fibroblasts (NF) were isolated from prepuce tissue. All fibroblasts were grown in free-serum culture medium for 24 hours to collect conditioned medium (CM). Then, CM was supplemented to HT-29 CRC cells for 72 hours. The effects of CM-T on the mRNA expression of OCT4 and ALDH1A1 were analysed using qRT-PCR. Supplementation of CM-T significantly increased OCT4 and ALDH1A1 mRNA expressions compared to

that of CM-NT, CM-NF, and control. We conclude that secretomes from CRC patients upregulate the expression of CRC stemness.