Induksi apoptosis dan angiogenesis oleh sCD40L dan aktivitas apoptosis oleh TNFα pada sel progenitor hematopoesis dan sel mesenkim sindrom mielodisplasia in vitro = sCD40L induced apoptosis, and angiogenesis also TNFα-induced apoptosis from hematopoietic progenitor cells and mesechimal cells of myelodysplastic syndromes in vitro.

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Abstrak

Fenomena utama pada pasien MDS adalah sitopenia di darah tepi, namun disertai kondisi hiperselular di sumsum tulang. sCD40L dianggap sebagai sitokin yang dapat memicu sintesis TNFα sebagai sitokin proapoptosis dan memicu sintesis VEGF sebagai sitokin proangiogenesis pada MDS. Oleh sebab itu sCD40L dianggap berpotensi sebagai biopenanda untuk memperkirakan perburukan pada MDS.
Penelitian ini bertujuan untuk membuktikan peran pajanan rh-sCD40L dalam menginduksi sintesis TNFα dan VEGF pada sel progenitor hematopoesis, serta membuktikan peran pajanan TNFα dalam memicu apoptosis pada sel progenitor hematopoesis dan sel mesenkim MDS.
Penelitian ini merupakan penelitian eksperimental in vitro komparatif. Subjek penelitian adalah pasien MDS yang didiagnosis dan diklasifikasikan berdasarkan kriteria WHO 2008. Pada bone marrow mononuclear cells (BMMC) dipajankan dengan rh-sCD40L dan antiCD40L, kemudian dilakukan pemeriksaan ekspresi mRNA TNFα dan mRNA VEGF yang dikuantifikasi dengan qRT-PCR, serta pemeriksaan kadar TNFα dan VEGF yang diperiksa dengan metode ELISA. Pada sel CD34+, CD33+, CD41+, dan CD73+ dipajankan rhTNFα kemudian dilakukan pemeriksaan aktivitas kaspase-3 dengan imunoflowsitometri.
Terdapat 15 sampel MDS terdiri dari 4 dengan diagnosis RCUD, 7 RCMD, dan 4 RAEB1, serta 7 sampel kontrol. Pajanan rh-sCD40L meningkatkan ekspresi mRNA TNFα secara bermakna dibandingkan pajanan antiCD40L. Pajanan rh-sCD40L meningkatkan kadar TNFα secara bermakna dibandingkan kontrol. Namun pajanan rh-sCD40L tidak meningkatkan mRNA VEGF dan kadar protein VEGF. Pajanan rhTNFα meningkatkan aktivitas kaspase-3 pada sel progenitor MDS terutama yang berdiferensiasi menjadi mieloid (CD33+) dan megakariosit-trombosit (CD41+). Pajanan rhTNFα meningkatkan aktivitas kaspase-3 pada sel mesenkim (CD73+) MDS
Simpulan: sCD40L berperan dalam meningkatkan sintesis sitokin proapoptosis TNFα di level mRNA dan protein, namun tidak terbukti berperan dalam meningkatkan sintesis proangiogenesis VEGF. TNFα berperan dalam meningkatkan apoptosis terutama pada sel hematopoesis yang telah berdiferensiasi menjadi seri mieloid dan seri megakariosit-trombosit, dan berperan dalam meningkatkan apoptosis pada sel mesenkim.
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Cytopenia is the primary phenomenon in Myelodysplastic Syndrome (MDS) patients, amidst hypercellular bone marrow. The soluble CD40 ligand (sCD40L) is considered as a cytokine that can trigger synthesis of TNFα and VEGF. The former is known as a cytokine that promotes apoptosis while the latter promotes angiogenesis in MDS patients. Therefore, the sCD40L may serve as a potential biomarker to

predict worsening of MDS.
This study aims to prove the role of rh-sCD40L exposure in inducing the synthesis of TNFα and VEGF in hematopoietic progenitor cells, as well as to establish the role of TNFα exposure in triggering apoptotic activity in hematopoietic progenitor and mesenchymal cells of MDS.
The study was a comparative in vitro experimental study. Subjects were MDS patients diagnosed and classified using the WHO 2008 criteria. Bone marrow mononuclear cells (BMMC) were exposed to rh-sCD40L and antiCD40L. The expressions of TNFα and VEGF mRNAs were then quantified by qRT-PCR, and the level of TNFα and VEGF were measured using the ELISA method. The CD34+, CD33+, CD41+, and CD73+ cells were exposed to rhTNFα, then the activity of enzyme caspase-3 was measured using the immunoflowcytometry.
There were 7 control and 15 MDS samples with the following diagnoses: 4 RCUD, 7 RCMD, and 4 RAEB1. Compared to antiCD40L, it is found that exposure of rh-sCD40L significantly increased the expression of TNFα mRNA. The similar exposure also significantly increased the level of TNFα compared to controls. However, the exposure of rh-sCD40L did not increase the expression of VEGF mRNA as well as the level of VEGF. The exposure of rhTNFα was found to increase the activity of caspase-3 in MDS progenitor cells, particularly those differentiated into myeloid cells (CD33+) and megakaryocyte-thrombocyte cells (CD41+). The exposure of rhTNFα was found to increase the activity of caspase-3 in MDS mesenchymal (CD73+) cells.
Conclusion: The sCD40L plays a role in increasing the synthesis of TNFα which favors apoptotic activity in mRNA and protein level, but not in improving the synthesis of VEGF that promotes angiogenesis. Furthermore, TNFα plays a role in increasing apoptotic activity of hematopoietic cells, particularly those that have differentiated into myeloid series and megakaryocyte-thrombocyte series cells. Also TNFα plays a role in increasing apoptotic activity of mesenchymal cells.