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Biological roles and activities of N – linked glycosylation site addition at Asn[69] of Dimeric EPO

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Human Erythropoietin (EPO) is a glycoprotein that is synthesized mainly in the kidney and that stimulates erythropoiesis through actions on erythroid progenitor cells. Early studies indicate that the liver is the primary site of production of EPO in the fetus, and there is gradual shift from the liver to the kidney shortly after birth. EPO is glycoprotein with a molecular mass of approximately 30 kDa, active hEPO consists of a single 165-amino acid polypeptide chain with three N-glycosylation sites a Asn^{24}, Asn^{38}, and Asn^{83}, respectively, and one O-glycosylation site at Ser^{126}.

We have been investigating the roles of glycosylation site addition in the biosynthesis and function of EPO (RDB, 2005 meeting, Purevjargal et, al; poster 29;PO584). Expression vectors for rec-hEPO were constructed according to the method previously described (RDB, 2005, 29:2). In this study, we investigated the roles and activities of dimeric-rechEPO (EPOWT, EPOΔ69, EPO Dimer, EPOWT+Δ69, EPOΔ69+WT and EPOΔ69+Δ69). The mRNA expression of rec-hEPO in CHO cell lines was detected by the Northern blot. we assayed the growth and differentiation of EPO-dependent human leukemic cell line (F36E) were used to measure cytokine dependency and *in vitro* bioactivity of rec-hEPO. MTT assay values were increased by survival of F36E cells at 24h. To analyze the *in vivo* biological activity, mice were injected subcutaneously with 10 IU per mice of rec-hEPO-derived molecules on days 0 and 2. Red blood cell and hematocrit values were measured 6

days after the first injection. The pharmacokinetics behavior was analyzed by the injection of 2.5 IU of rec-hEPO.

The result of Northern blot was expressed in all EPO cell lines. Dimeric EPO samples had a slightly higher band than WT or Δ69. The MTT assay result showed higher value than WT EPO in F-36E cells for EPO dependent cell proliferation. The hematocrit values remarkably increased in all treatment groups. Especially, the EPOΔ69+WT, Δ69+Δ69 groups were enhanced. The pharmacokinetics result was peak at 2h after injection in all groups. Δ69 mutant was the highest peak (about 2,000 mIU/ml) at 2 h after injection and WT+Δ69, Δ69+WT was highly detected. However, it was almost similar pattern between dimeric and Δ69+Δ69. The long half-life of rec-hEPO mutants is likely to confer clinical advantages by allowing less frequent dosing in patients treated for anemia.

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