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COMP-Ang1 Recovers Hematopoiesis after Gamma-ray Irradiation in Mice

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1. Introduction

It has been reported that angiopoietin (Ang1) enhanced the ability of hematopoietic stem cells (HSCs) to become quiescent and induced adhesion to bone, resulting in protection of the HSC compartment from myleosuppressive stress [1]. Ang1 activates Tie2 receptors expressed on the vascular endothelial cells [2] and HSCs expressing the receptor tyrosine kinase Tie2 are quiescent and antiapoptotic, comprise a side-population of HSCs [1]. COMP-Ang1 is generated protein, is a soluble, stable, and potent Ang1 variant by replacing the N-terminal portion of Ang1 with short coiled-coil domain of cartilage oligomeric matrix protein (COMP) [3]. Radio- and chemotherapy induces considerable damage of bone marrow [4]. Herein, by using COMP-Ang1, we investigated the effect of COMP-Ang1 on restoration of hematopoiesis in bone marrow against radiation-induced myelosuppression.

2. Methods and Results

Animals

C57BL/6 female mice, six in each group, aged 6 to 8 weeks were provided by SCL. Animals were kept under in conventional condition with free access to water and food.

Irradiation and virus infection

C57BL/6 mice were randomly assigned into five groups: normal control group, control adenovirus treated group, Ad COMP-Ang1 treated group, radiation control group, Ad COMP-Ang1 treated and irradiated group. At 24 hrs after Ad COMP-Ang1 intravenously injection ($1x10^9$ pfu /head), experimental mice were irradiated by 4.5 Gy from a ^{60}Co gamma-ray source.

Spleen colony forming assay

To enumerate spleen colony forming Ad COMP-Ang1 was transferred intravenously via the lateral tail vein and irradiated. The mice were killed after 9 days and their spleen were removed to Bouin's solution. Individual surface colonies, each comprising the progeny of a single stem cell, were counted and the numbers of colonies per mouse were calculated by gross examination.

Sample analysis

Samples obtained at 1, 4, 7, 14, and 21 days following IR. Bone marrow cell counts were performed from left femur medulla of each mouse. Suspension of mouse bone marrow cells were obtained by flushing RPMI 1640 through excised femurs. The cells were treated with NH_4Cl (0.184 mol/L) to lyse erythrocytes, washed, and resuspended in RPMI for counting and assessment of viability by trypan blue exclusion. Each right femur was fixed by formalin, decalcified and then embedded in paraffin and sectioned at 3-4 ㎛. The sections were stained with hematoxylin-eosin, examined for evidence of pathological changes and measured bone marrow cellularity by using image analyzer, and processed for immunohistochemistry of Tie2 expression in bone marrow HSCs.

3. Results

At spleen colony forming assay, COMP-Ang1 treated mice were shown significant increases of colonies forming [17.8 ± 8.58, $p<0.05$] compared with control virus injected mice [6.2 ± 3.83]. After irradiation, bone marrow HSCs were significantly decreased and slowly restored. At histopathologic analysis, COMP-Ang1 showed rapider restoration of HSCs than control virus administrated groups at 4, 7, 14 and 21 days (Figure 1). When we measured Tie2 expression in bone marrow by immunohistochemistry, there were an increase in Tie2 positive HSCs in Ad COMP-Ang1 transferred mice when compared to control virus treated group (Figure 2).

These results indicated that the administration of COMP-Ang1 to mice leads to significant recovery of bone marrow hematopoiesis and protection of radiation induced damage.

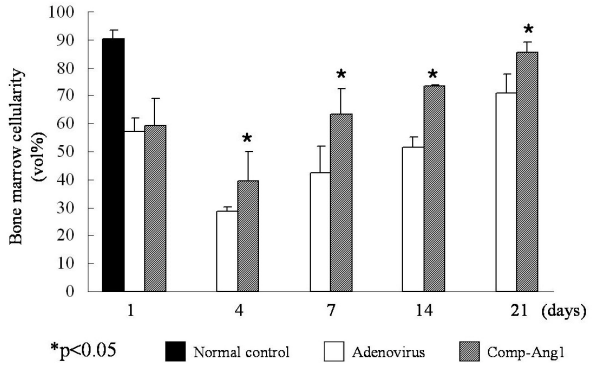

Figure 1. Recovery HSCs in bone marrow during post irradiation days.

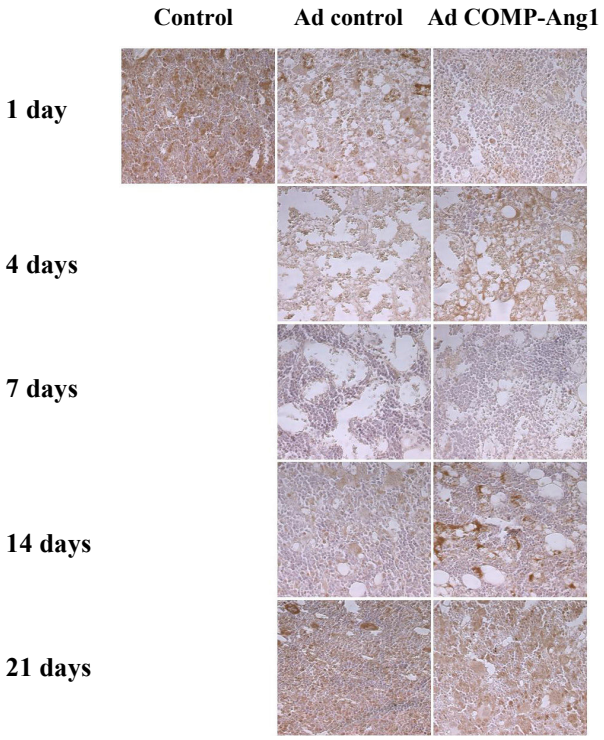

Figure 2. Tie2 expression in bone marrow during post irradiation days.

4. Conclusion

Our studies have shown that COMP-Ang1 administration has a function of recovery and protection of hematopoiesis in radiation induced damage *in vivo*. These results suggested that COMP-Ang1 could be used as a therapeutic protein for specific protection against radiation induced HSCs injury.

References

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