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Relationship between Rac1 levels and neutrophil counts in myelodysplastic syndrome patients

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Myelodysplastic syndrome (MDS) is a heterogenous group of clonal hematopoietic disorders characterized by ineffective hematopoiesis that results in refractory cytopenia in spite of hyper cellularity in bone marrow, and morphological and functional abnormalities in single or multiple lineages [1,2]. Numerical and functional reductions of neutrophilic granulocytes are associated with high mortality of infections [3,4]. However, the molecular basis of ineffective hematopoiesis is largely unclear. Since the median age of diagnosis is 65-70 years, and exposure to irradiation and treatment with anti-cancer drugs can cause MDS [5], aging and genotoxic stress are considered to be involved in the development of MDS.

We recently found that decreases in Rac1, a member of the Rho GTPase family, as well as in DOCK8 and FGD4, two activators of another Rho GTPase member, Cdc42, contributed to impaired neutrophil migration in MDS [6]. When the average of expression levels quantified by Western blotting in healthy individuals was set at 1.0, Rac1, DOCK8, and FGD4 levels in MDS neutrophils varied from 0.61 to 1.16, 0.13 to 0.47, and 0.28 to 1.11, respectively.

The Rho GTPase family is a group of intracellular signaling proteins that regulate many important cellular functions, including transcription, survival, adhesion and cytoskeleton reorganization [7]. Most Rho GTPases switch from inactive GDP-bound to active GTP-bound forms by upstream guanine nucleotide exchange factors (GEFs). Among 22 Rho GTPase members, Rac1 and Cdc42 have been extensively studied. Recent mouse model studies have demonstrated that aging is associated with elevated Cdc42 activity in hematopoietic stem cells, which induces a relative expansion of myeloid-biased HSCs over lymphoid-biased HSCs, decline in survival, adhesion, and engraftment of HSCs [8]. Therefore, we speculated that the reduction in DOCK8 and FGD4, but not Rac1, influences blood cell counts in MDS patients by attenuation of Cdc42 activity.

Neutrophils isolated from 10 MDS patients were subjected to quantification of DOCK8, FGD4, and Rac1 by Western blotting. We then analyzed whether their expression levels were correlated with any of four hematological parameters: white blood cell counts, neutrophil counts; hemoglobin concentration, and platelet counts in peripheral blood.

As shown in Table 1, neither DOCK8 nor FGD4 levels had significant correlations with any of the hematological parameters tested. In contrast, a significant positive correlation was detected between neutrophil counts in blood and Rac1 ($r = 0.745$, $p < 0.05$). Other hematological parameters, including total white blood cell counts, were not correlated with Rac1 levels. This result raised several questions: (1) whether low Rac1 expression is causative to neutropenia in MDS; (2) what the consequences of low Rac1 expression in neutrophils are; (3) if Rac1 is downregulated in hematopoietic stem cells and progenitors, how Rac1 reduction affects hematopoiesis; and (4) what determines Rac1 protein levels in MDS neutrophils.

Table 1. Correlation coefficients

	Neutrophil counts	White blood cell counts	Hemoglobin concentration	Platelet counts
DOCK8	-0.482 ($p = 0.133$)	-0.326 ($p = 0.328$)	-0.099 ($p = 0.771$)	0.411 ($p = 0.209$)
FGD4	-0.072 ($p = 0.844$)	-0.030 ($p = 0.934$)	0.046 ($p = 0.899$)	0.373 ($p = 0.289$)
Rac1	0.745 ($p = 0.013$)	0.564 ($p = 0.089$)	0.441 ($p = 0.202$)	0.183 ($p = 0.614$)

Egress of neutrophils from bone marrow may depend on Rac1 in MDS, although Rac1 is not a single determinant of neutrophil release from bone marrow. To date, neutropenia has not been demonstrated by studies using dominant negative mutant or gene targeting of Rac1, while reduction in F-actin polymerization and chemotaxis of neutrophils have been observed [9]. It is of interest whether other neutropenic disorders also exhibit correlations between Rac1 levels in neutrophils and their counts in circulation.

Homing of neutrophils to bone marrow may also be suppressed by Rac1 reduction in neutrophils. Recent studies have unveiled CXC4-mediated homeostatic roles of neutrophils [10,11]. Compared with neutrophils newly-released from bone marrow, aged neutrophils increase expression of CXCR4, a receptor for CXCL12 that allows their homing to bone marrow from blood. Infiltration of aged neutrophils reduces the size and function of hematopoietic niche, resulting in release of progenitor cells from bone marrow through phagocytosis by medullary macrophages and activation of Liver X Receptors [10]. CXCR4 and CXCL12 interaction-initiated migration is mediated through Rac1 [12]; therefore, both reduction of neutrophils in circulation and downregulation of Rac1 in neutrophils are likely to inhibit neutrophil homing, which mitigates progenitor release and increases cellularity in bone marrow.

If Rac1 levels in hematopoietic stem cells and progenitors are parallel to those in neutrophils, it would influence the genotoxic stress response of hematopoietic cells in bone marrow. Rac1 is a key regulator of stress-activated protein kinases (SAPK/JNK), p38 kinase and extracellular regulated kinases (ERK) as well as numerous

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transcription factors, including NF-κB and AP1 [13-15]. Although DNA damage response following double strand breaks of DNA is cell type- and genotoxic agent-specific, various roles of Rac1 in DNA damage response (DDR) have been reported. Rac1 enhances the binding of anthracyclines to their target Topoisomerase II, resulting in DSB formation and activation of DDR in cardiomyocytes [16]. In hepatocytes exposed to anthracyclines, the absence of Rac1 enhanced γH2AX levels, pro-fibrotic factors, such as TGF-β, and senescence marker p16 in the subacute phase, while acute toxicity was mitigated [17]. Senescence-associated features of hematopoietic cells with heterogenous expression of DDR markers observed in MDS [18,19] may be related with insufficient expression of Rac1.

The regulators that influence Rac1 expression in neutrophils are not yet fully understood. One of the negative regulators known is miR-155. We previously reported that miR-155 was increased in the neutrophils of about two-thirds of MDS patients [20], and that overexpression of miR-155 suppresses Rac1 levels [6]. The correlation between miR-155 and Rac1 in MDS patients, however, was not statistically significant [6], suggesting the involvement of other Rac1 regulators. On the other hand, a recent study demonstrated that miR-25 was a positive regulator of Rac1 [21]. Aberrant expression of miR-25, which was previously shown to be downregulated in mononuclear cells isolated from MDS bone marrow [22], may contribute to reduction of Rac1.

The relevance of Rac1 reduction to functions of neutrophils as a hematopoietic homeostasis regulator and granulopoiesis may be a crucial point to understand the mechanisms of developing ineffective hematopoiesis in MDS.

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