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Rest as a New Transcription Factor to Control Neural Crest Development

Hitomi Aoki and Takahiro Kunisada*

Department of Tissue and Organ Development, Regeneration, and Advanced Medical Science, Gifu University Graduate School of Medicine, Gifu, Japan

***Corresponding author:** Takahiro Kunisada, Department of Tissue and Organ Development, Regeneration, and Advanced Medical Science, Gifu University Graduate School of Medicine, 1-1 Yanagido, Gifu 501-1194, Japan, Tel: +81-58-230-6477; Fax: +81-58-230-6478; E-mail: tkunisad@gifu-u.ac.jp

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Abstract

RE1-silencing transcription factor (Rest), also known as NRSF (neuro-restrictive silencer factor), is a negative regulator of neuron-specific genes and expressed during embryonic development to prevent neural gene expression in non-neuronal cells. However, Rest null mice die by E11.5, prior to which the growth retardation caused by widespread apoptotic cell death has precluded further analyses of the potential role of Rest *in vivo*. In order to investigate the function of Rest in neural crest cells (NCCs) which are known to differentiate into neuronal and non-neuronal lineages, we established NCC-specific homozygous Rest conditional knockout (CKO) mice and observed their neonatal death caused by the defect of enteric nerve cells derived from NCCs. The viable heterozygous NCC-specific Rest CKO mice showed the white spotting phenotype, associated with a reduction in the number of melanoblasts, a non-neuronal derivative of NCCs, in embryonic skin. These results suggest the expression of REST during the early NCC specification stage is necessary for the proper development of NCCs. To fully understand the mechanisms of white spot formation and postnatal death or embryonic lethality mediated by the Rest ablation, future experiments should focus on single cell analysis to characterize the detailed cellular events such as reduced cell cycle, apoptosis, change of the cell fate to well explain the observed phenotypic changes.

Keywords: Rest/NRSF; Neural crest cells; Melanocytes; Conditional knockout mouse

Commentary

RE1-silencing transcription factor (REST), also known as NRSF (neuron-restrictive silencer factor), is a zinc finger protein that binds to a conserved 23 bp motif known as RE1 (repressor element, also called NRSE) found in more than 1000 of genes determining the fundamental neuronal traits [1,2]. Rest is working in undifferentiated stem cells as well as during neural maturation [3-5]. During embryonic stem cell (ESC) differentiation, Rest expression is highest in undifferentiated ESCs and is down-regulated as the ESCs differentiate into neuronal stem cells (NSCs) then completely silenced in mature neuronal cells [6].

Unfortunately, Rest KO mice showed embryonic lethality and these observations have been tested only by *in vitro* culture system. To investigate the Rest function *in vivo*, Rest conditional knockout (CKO) mice carrying the floxed last exon of Rest encoding the coRest binding site, essential for the construction of Rest silencing complex was developed [7,8].

By using this conditional knockout (CKO) system, Rest was shown to promote the early differentiation of ESCs by silencing Nanog expression during the early differentiation of ESCs *in vitro*. Nanog is a member of core transcription factor in the maintenance of ESC pluripotency and harbors RE site in its promoter. It was also shown that Rest is not required for the maintenance of undifferentiated state of ESCs [9]. Then Rest was shown to play a role in suppressing the expression of neuronal genes in cultured neuronal cells. Surprisingly, by using Rest CKO mice, neuronal genes were also suppressed in non-neuronal cells outside of the central nervous system, however, Rest function is still dispensable *in vivo* for the progression of embryonic neurogenesis [10]. Causative Rest aberration has been reported in patients with Alzheimer's disease [11], microcephaly [12], epileptic seizures [13], Huntington's Disease [14], Down's syndrome [15,16]. In these neurodegenerative disorders, Rest aberration induced the aberrant expression of various genes including the target neuronal genes. In addition to the neurogenesis, recent studies have suggested that Rest modulates glial lineage elaboration by coupling neurogenesis and gliogenesis [17,18], and the breakdown of these processes accompanies neurodegenerative disorders.

Neural crest (NC) cells emerge from the dorsal region of the neural tube of the vertebrate embryos and migrate throughout the body. They then differentiate into many cell types, including neurons and glial cells of the peripheral sensory and autonomic ganglia, Schwann cells, melanocytes, endocrine cells, smooth muscle cells, and skeletal and connective tissue cells of the craniofacial complex [19]. The multipotential cell fate of NC cells to differentiate into neuronal and non-neuronal lineages urged us to investigate the relationship of the NC cell fate determination and the REST function.

To accomplish this, we generated NC cell specific Rest CKO mice by crossing NC cell specific Wnt1-Cre driver containing transgenic mouse with Rest floxed mouse that allowed us to examine the effects of genetic ablation of Rest during *in vivo* NC cell differentiation [20]. These Rest CKO mice showed neonatal but not embryonic lethality that was characterized by gastrointestinal tract dilation caused by the reduced acetylcolinesterase gene expression appeared in the NC cell-derived myenteric plexus, despite their intact morphology. Thus, strong evidence was obtained for REST to properly maintain a specific NC derived cell lineage, which may imply the widespread REST function in various NC cell lineages.

Melanocyte is one of the non-neuronal NC cell lineages and supplies melanin pigments to developing hair. We observed the expanded berry white spots in viable heterozygous NC cell-specific Rest CKO mice

[21]. This white spotting phenotype was associated with the reduction in the number of melanocytes in the embryonic skin. However, the Rest deletion induced after the specification to melanocytes by using melanocyte specific Tyr-Cre or DCT-Cre driver did not reduce the number of melanoblasts; therefore, the expression of REST during the early neural crest specification stage was necessary for the normal development of melanoblasts to cover entire skin. Our results indicate that NC cell fate is regulated, at least in part, by Rest directing specification of multipotent NC cell to the melanocyte cell fate.

Now we are investing the precise function of Rest on NC cell differentiation, especially for the mechanisms to modulate specification of melanocyte cell fate from multipotent NC stem cells. This may finally explain the observed white spotting phenotype in NC cell-specific Rest CKO mice and illuminate the novel function of Rest.

Extremely large numbers of the Rest target genes might imply that some specific phenotypes are not the goal of Rest biology. Establishing Rest CKO mice one by one by using various Cre driver constructs as introduced here and comparing the phenotypes and underlying changes of the gene expression pattern is slow but feasible strategy to reveal the whole picture of Rest biology (Figure 1).

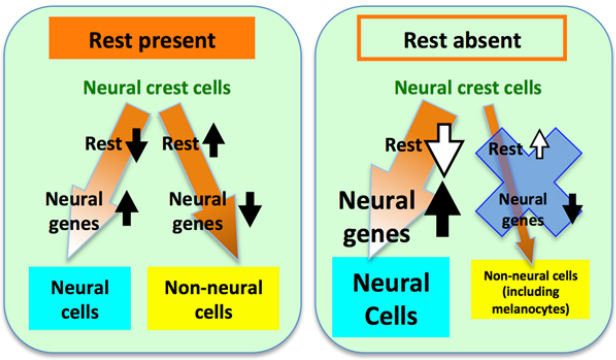

Figure 1: Rest controls differentiation of neural crest cells. In neural crest cell differentiation, Rest expression decreases in the cells taking neural cell fates. On the other hand, non-neural cells consistently express Rest to prevent the expression of neural genes (left). Without Rest, neural crest cells are more inclined to take neural cell fate (right).

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