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REVIEW

ECRG4: a new potential target in precision medicine

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Abstract **Given the rapid development in precision medicine, tremendous efforts have been devoted to discovering new biomarkers for disease diagnosis and treatment. Esophageal cancer-related gene-4 (*ECRG4*), which is initially known as a new candidate tumor suppressor gene, is emerging as a sentinel molecule for gauging tissue homeostasis. ECRG4 is unique in its cytokine-like functional pattern and epigenetically-regulated gene expression pattern. The gene can be released from the cell membrane upon activation and detected in liquid biopsy, thus offering considerable potential in precision medicine. This review provides an updated summary on the biology of ECRG4, with emphasis on its important roles in cancer diagnosis and therapy. The future perspectives of ECRG4 as a potential molecular marker in precision medicine are also discussed in detail.**

Keywords ECRG4; tumor suppressor gene; sentinel molecule; precision medicine; cell senescence; epithelium homeostasis

Introduction

Current cancer diagnosis and treatment in the clinic is not ideal given that diagnostic tests are usually invasive and treatments are "one-size fits-all" instead of being customized. Accelerated new discoveries and technologies in medicine have made precision medicine possible, but new emerging targets are still required. Targets in precision medicine cover a wide range of biochemical entities, such as proteins, nucleic acids, ncRNAs, sugars, small metabolites, cytogenetic and cytokinetic parameters, and circulating tumor cells found in body fluid [1].

Among these entities, esophageal cancer related gene-4 (*ECRG4*) is a potential target that was originally cloned and identified by Su and colleagues from normal human esophageal epithelium in 1998 [2]. Increasing data have shown that the expression of *ECRG4* is correlated with numerous diseases such as cancer, aging, and injury, thereby indicating its considerable potential as a new target in precision medicine. In this review, we summarize the major findings on ECRG4, including its molecular features and its interesting physiological and pathological roles, and discuss the potential of ECRG4 as a target in precision medicine.

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ECRG4: gene, protein, processing and pathway

The *ECRG4* gene, also called *C2ORF40*, is localized on chromosome 2q12.2 [3]. The gene consists of four exons and is highly conserved among species, thereby indicating that *ECRG4* may perform essential roles *in vivo* [4]. Bioinformatic analysis predicted that the *ECRG4* encodes a 17 kDa protein, which contains a leader peptide at residues 1–30, a putative furin-like cleavage site at residues 68–71, and a predicted thrombin cleavage site at residues 130–134 [5,6].

Similar to transforming growth factors or tumor necrosis factors, which are cell membrane proteins that are released after cell surface processing [7,8], the full-length ECRG4 can be processed to multiple peptides either continually located on cell membrane or released from cell membrane, depending on the cell types [9]. Thus, a minimum of 10 forms of ECRG4 can theoretically be produced after different processes. Thus far, 17, 14, 10, 8, 6, 4, and 2 kDa ECRG4-derived peptides have been identified, corresponding to ECRG4 (residues 1–148), augurin (residues 31–148), CΔ16-augurin (residues 31–130), argilin (residues 71–148), CΔ16-argilin (residues 71–130), and CΔ16 (residues 134–148) [3,5] (Fig. 1). Different from numerous other tumor suppressor genes, such as *p53* and *Rb1*, which usually encode intracellular proteins that serve as transcription factors or components of intracellular signaling pathways, the candidate tumor suppression gene *ECRG4* is

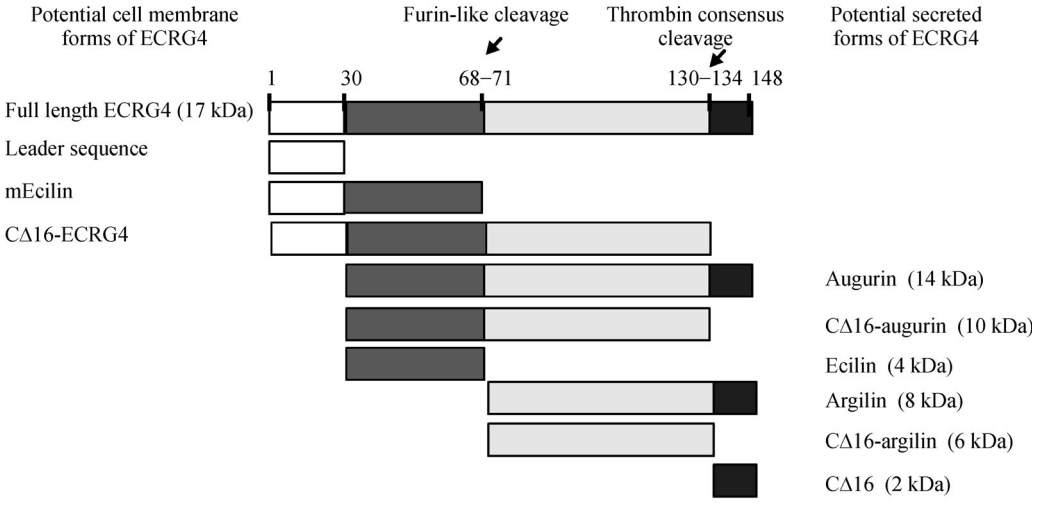

Fig. 1 Processing of ECRG4 protein. The ECRD4 gene encodes a 148-amino-acid (aa) protein that consists of 30-aa leader sequence and 14 kDa augurin. ECRG4 presumably contains two consensus protease cleavage sites. One furin-like cleavage site is located at 68–71, and the other thrombin consensus cleavage site is located at 130–134. ECRG4 processing from furin-like cleavage site may yield two peptides, namely, ecilin (4 kDa) and argilin (8 kDa); processing from the thrombin consensus cleavage site may yield three peptides, namely, CΔ16, CΔ16-augurin, and CΔ16-argilin.

considered as a cytokine- or chemokine-like growth factor, which exerts its function in an autocrine or paracrine approach [10,11].

The expression of *ECRG4* is strictly regulated. A considerable distribution of CpG islands in the promoter region of *ECRG4* gene, as revealed by bioinformatics analysis and the methylation of ECRG4 promoter, is a key factor that regulates the expression of ECRG4 [12]. Methylation inhibitors, such as 5-AZA-C, can increase *ECRG4* expression *in vivo* and *in vitro* presumably by inhibiting DNA methylation [13]. Thus far, few works have revealed the downstream molecules regulated by ECRG4. Li found that transfection of *ECRG4* gene in ESCC cells can increase the expression of p53 and p21 and induce cell cycle G1 phase block. It was also reported that ECRG4 may exert its function by regulating NF-κB and COX-2 [14,15] (Fig. 2).

ECRG4 in physiology and pathology

Immunohistology and reverse transcription PCR (RT-PCR) on tissue samples reveal that *ECRG4* is widely expressed in human and rat tissues [16,17]. However, the exact cell types expressed in each tissue should be further identified with more precise approaches such as confocal microscopy or RT-PCR on sorted cells. After 20 years since the discovery of ECRG4, its crucial roles in inducing cell senescence, homeostasis guarding, and anti-tumor effect are gradually identified. These roles are discussed comprehensively in the following section.

ECRG4 in cell senescence

Mirabeau *et al.* identified ECRG4 as a novel candidate peptide hormone through a bioinformatic approach using hidden Markov model formalism in 2007 [3]. Further biochemical analysis showed that ECRG4 is localized in secretory granules and can be recovered from the supernatant. Three years later, the work of Kujuro identified ECRG4 as a senescence inducer with implications for the senescence-like state of postmitotic cells in the aging brain [18]. The study started from the concept that ECRG4 emerged as the largest changed molecule between the senescent and nonsenescent oligodendrocyte precursor cells (OPCs) by using DNA microarray analysis. As shown by the study, ECRG4 is upregulated in senescent OPCs; its overexpression in OPCs induces senescence, and its knockdown by a specific short hairpin RNA prevents these phenotypes. Moreover, increased ECRG4 expression was observed in OPCs and neural precursor cells in the aged mouse brain, accompanied by the expression of senescence-associated β-galactosidase activity, thereby indicating the cells' entry into senescence. These data indicate that ECRG4 is an autocrine factor that guards neural-cell senescence.

ECRG4 in epithelium homeostasis upon infection, inflammation, and injury

ECRG4 is assumed to have constitutive functions that maintain homeostasis because of the wide tissue distribution. Thus far, ECRG4 has been mainly expressed on

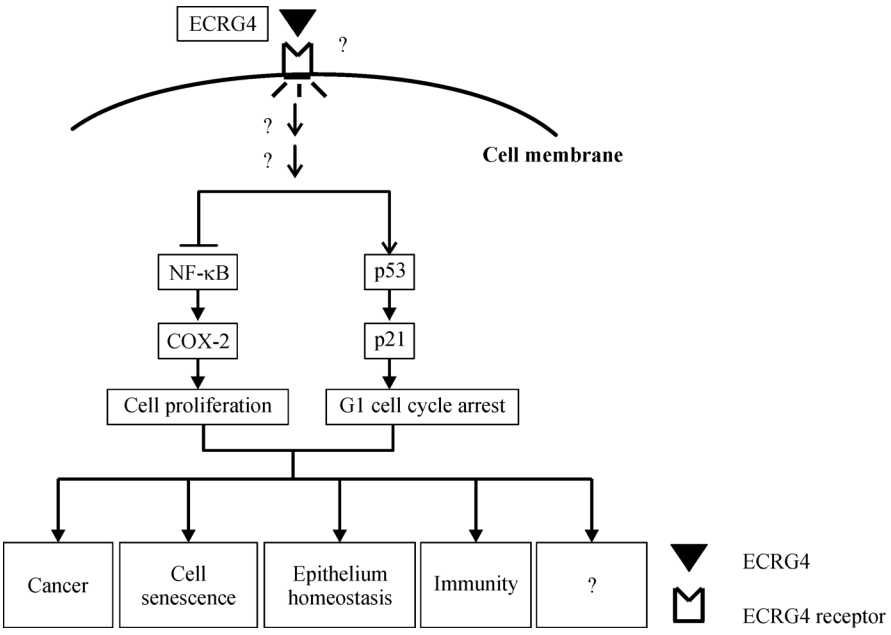

Fig. 2 Roles of ECRG4 *in vivo* and its underling molecular mechanism. After binding to undefined ligands on the cell membrane, ECRG4 stimulates downstream cascades to affect cell proliferation or G1 cell cycle arrest by employing p53 pathway or inhibiting NF-κB-COX-2 signaling pathway. ECRG4 plays important roles in cancer, cell senescence, homeostasis guarding, and immunity and other undiscovered functions represented by "?".

epithelial cells, including specialized epithelial-derived cells and even hematopoietic cells. The sentinel functions are only beginning to emerge.

Baird *et al.* found that choroid plexus epithelia are a major source of ECRG4 in the CNS [13]. *ECRG4* gene expression sharply decreases after a stab injury into the brain. The loss of ECRG4 is circumvented by *in vivo* overexpression, and BrdU incorporation by cells in the subependymal zone decreases. Inversely, gene knockdown of *ECRG4* in developing zebrafish embryos causes augmented proliferation of glial fibrillary acidic protein-positive cells and induces a dose-dependent hydrocephalus-like phenotype. However, co-injection of antisense morpholinos with ECRG4 mRNA can rescue this phenotype [6]. Furthermore, in another study with a traumatic brain injury rat model, dynamic expression of *ECRG4* in CNS injury was observed, demonstrating that *ECRG4* gene expression and augurin protein levels decreased at 24–72 h post-injury but restored to uninjured levels by day 7 post-injury. These experiments established a causal relationship between the decrease in *ECRG4* expression and injury-induced changes, thereby suggesting that ECRG4 may play constitutive inhibitory function in normal CNS, whereas the downregulated *ECRG4* expression in injury encourages proliferation [19].

A series of work further subsequently revealed the dynamic expression of *ECRG4* on epithelia-oriented cells upon infection, inflammation, or injury and the role of ECRG4 as a growth inhibitor. Kurabi *et al*. found that post-infection constitutively expressed ECRG4 on normal quiescent ME mucosa is rapidly downregulated [20]. Overexpression of *ECRG4 in vivo* prevents the natural downregulation of *ECRG4*, reduces mucosal proliferation, and prevents inflammatory cell infiltration that is normally observed after infection. Kao *et al.* also demonstrated that ECRG4 is characteristically downregulated in human lung epithelial cells following inflammatory lung injury and that the overexpression of *ECRG4* in human lung epithelial cells *in vitro* decreases cell proliferation [12]. Similar responses were observed in acute cutaneous injury and other chronic inflammation [21]. In summary, *ECRG4* is constitutively expressed, but its expression decreases rapidly following extinct stimulations immediately preceding cell proliferation. The recovery of *ECRG4* gene expression also precedes the return to quiescence. These results implied that ECRG4 may play an important role in coordinating the inflammatory and proliferative response to maintain epithelium homeostasis.

ECRG4 in immunity

Baird *et al.* found that *ECRG4* is markedly more highly expressed (600–800 times) in human PBMCs compared with cultured cell lines. Full-length ECRG4 is localized on PMN and monocyte cell surfaces, and LPS treatment can induce the release of ECRG4 from the cell surface [13]. The loss of cell surface ECRG4 is associated with inflammatory response that follows a severe, cutaneous

burn injury, as further confirmed by Costantini *et al.* in a burn injury patient population [22]. Podvin *et al*. also reported that ECRG4 is present on the surface of human monocytes and granulocytes. Furthermore, the interaction between ECRG4 and the human innate immunity receptor complex was discovered, supporting a role for cell surface activation of ECRG4 during inflammation [23]. In addition, incubation of macrophages with a soluble ECRG4-derived peptide increased p-p65 phosphorylation, thereby suggesting that processing of an intact sentinel ECRG4 on quiescent circulating leukocytes leads to processing from the cell surface that follows injury and macrophage activation [13]. These results further support the imperative roles of ECRG4 as a homeostasis sentinel molecule.

ECRG4 in cancer

ECRG4 was discovered by Su *et al*. in their search for differentially expressed genes between esophageal cancer patient samples and normal controls [2]. They identified four novel genes, namely, *ECRG1–4*, which are either expressed in normal esophageal epithelia but absent in esophageal cancer or alternatively expressed in esophageal cancer but not detected in normal esophageal epithelia. Among them, *ECRG4* gene expression appeared unique, with decreased expression in tumor cells but readily detectable expression in normal tumor-adjacent tissue. A subsequent bioinformatic approach by Bi *et al*. supported a broad role for ECRG4 in the control of cancer cell growth [24]. Subsequently, a sudden increase in studies extended the anti-tumor roles of ECRG4 to a variety of cancers far beyond esophageal cancer, thereby earning its fame as a tumor suppression gene. The cancer cell lines were cultured *in vitro*, and the overexpression of *ECRG4* was found to promote the apoptosis and inhibit the proliferation of many cancer cells including colorectal cancer cells, human head and neck cancer cells, human laryngeal cancer cells, and even some immortalized cell lines such as Jurkat cells and HEK 293T cells [25–28]. The upregulation of *ECRG4* could also promote the migration and invasion of certain cancer cells, such as human breast cancer cell lines BT549 and MDAMB231 and glioma cell line U251 [29,30]. However, all studies were executed by over-expressing *ECRG4* in different cancer cell lines and with *in vitro* approaches; whether these finding apply *in vivo* awaits further intensive investigations.

The study of Lee *et al.* in 2015 set a milestone in the field. This work confirmed the crucial antitumorigenic role of ECRG4 with *ECRG4* knockout (KO) mice and the xenograft and syngeneic glioma models [31]. In terms of the cellular mechanism, ECRG4 promotes monocyte recruitment and activation of microglia in a T/B cell-independent mechanism, thereby resulting in reduced glioma tumor burden and increased survival. Tumor-induced myeloid cell recruitment is impaired in *ECRG4* KO mice, leading to increased tumor burden and decreased survival. These results evidently demonstrated that the anti-tumor effects of ECRG4 do not directly inhibit tumor cell growth; instead, ECRG4 may perform its anti-tumor role by activating macrophages or recruiting monocytes to promote the pro-inflammatory effect. Moriguchi *et al*. also confirmed that ECRG4 contributes to the anti-glioma effect through immune-surveillance via type-I interferon signaling [32]. These results are consistent with the previous study that stated that leukocytes are a rich natural source of ECRG4 and that a thrombin-processed, 16-amino-acid peptide is a chemoattractant of myeloid cells. Therefore, ECRG4 is a physiological immunomodulatory/immunosurveillance factor that regulates the tumor immune microenvironment and the control of tumor growth when introduced into the tumor bed.

ECRG4: a new potential target in precision medicine

ECRG4 as a non-invasive biomarker for diagnosis, prediction, and prognosis

The definitive anti-tumor effects of ECRG4 inspired the studies to explore whether the gene can be used as a biomarker for cancer diagnosis, prediction, and prognosis. ECRG4 possesses a cytokine-like functional pattern and is detectably in body fluid. Liquid biopsy from blood, urine, saliva, pleural effusions, and cerebrospinal fluid has gained considerable interest for developing the new diagnosis biomarker because liquid biopsy is easy and non-invasive to collect. Increasing data showed that the downregulation of ECRG4 is correlated with lymph node metastasis and predicts poor outcome in numerous cancers, such as esophageal carcinoma, breast cancer, prostate cancer, gastric cancer, nasopharyngeal carcinoma, and renal cell cancer [33–39]; hence, further attention is required (Table 1).

In addition, accumulating data indicated that methylated cDNA is a promising biomarker for diagnosis [41,42]. ECRG4 possesses an epigenetically-regulated gene expression pattern, and the promoter methylation status controls the expression of *ECRG4*. ECRG4 promoter hypermethylation can be detected in the peripheral blood of NPC patients, and aberrant ECRG4 promoter methylation may be used to monitor early cancer and predict pathological staging [37]. These findings indicated the potential value of ECRG4 as a non-invasive biomarker for cancer diagnosis, prediction, and prognosis (Table 2).

ECRG4 in developing new strategy for therapy

ECRG4 has been linked to a variety of diseases, including cancer, injury-related epithelium homeostasis, or aging;

Table 1 Functions of ECRG4 in cancer cells

Cell line name	Cell source	Regulation of ECRG4	Impacts of ECRG4	References
HCT116 and SW480	Colorectal cell lines	ECRG4↑	Cell proliferation↓ Cell viability ↓	[25]
SW480 and Caco-2	Colorectal cell lines	ECRG4↑	Cell proliferation↓ Cell apoptosis↑	[26]
M2 cell	Head and neck cells	ECRG4↑	Proliferation ↓ Cell cycle arrest ↑ Apoptosis↑	[27]
Hep-2 and LSC-1	Human laryngeal cancer cell lines	ECRG4↑	Cell proliferation↓ Cell cycle arrested in G0/G1↑	[28]
BT549 and MDAMB231	Breast cancer	ECRG4↑	Proliferation↓ Migration and invasion↓	[29]
SKBR3 and MDAMB468	Breast cancer	ECRG4↓	Growth↑ Invasion↑	[29]
U251	Glioma cells	ECRG4↑	Proliferation and inhibition of cell migration and cell cycle progression ↓	[30]
SGC-7901	Gastric cell line	ECRG4↑	Apoptosis↑ Sensitivity to 5-FU↑	[4]
CNE1 cell line	Human nasopharyngeal cancer cells	ECRG4↑	Cell growth rate↓ Sensitivity to cisplatin↑	[40]

Table 2 Clinic significances of ECRG4 in cancer

Cancer type	Expression of ECRG4	Clinic significance	References
Esophageal carcinoma	ECRG4↓ or none	Related to the degree of tumor invasion level, TNM staging, lymph node metastasis, and recurrence and survival after surgery	[33]
Breast cancer	ECRG4↓	Related to the tumor stage, tumor volume, risk of distant metastasis, survival rate, and overall survival time	[34,35]
Prostate cancer	ECRG4↓	Prostate-specific antigen recurrence Histology differentiation and lymph node metastasis	[36]
Gastric cancer	ECRG4↓	Cancer stages	[37]
Nasopharyngeal carcinoma	ECRG4↓	Correlated with lymph node metastasis and predicts poor outcome	[38]
Renal cell cancer	ECRG4↓	Associated with poor prognosis	[39]

thus, the gene becomes a potential target for therapeutic drug development (Table 2). First, for maximizing the anti-tumor effect, the upregulation of *ECRG4* expression and activity either alone or in combination with other cancer treatment strategies is worth intensive investigation. We manifested that the overexpression of *ECRG4* can enhance the responsiveness of gastric cancer cell line SGC-7901 to 5-FU and the responsiveness of human nasopharyngeal cancer cell line CNE1 to cisplatin [4,40]. Second, in other disease settings where the attenuated expression of *ECRG4* leads to tissue dysfunction, the upregulation of the expression or activity of ECRG4 may be valuable. For example, a recent study reports that the downregulation of ECRG4 is associated strongly with atrial fibrillation (AR) of patients [9], and whether or not ECRG4 can be used to treat AR is interesting to determine. Third, to fight against aging-related cell senescence, ECRG4 expression or activity should be downregulated.

In addition to directly overexpressing ECRG4 or using ECRG4 peptides, manipulating the expression of ECRG4 activity includes numerous approaches. *ECRG4* expression can be silenced by methylation of its promoter, which can be reactivated with demethylating agents. The two classes of demethylating agents include nucleoside DNMT inhibitors (DNMTi) and non-nucleoside DNMT inhibitors. The former class contains 5-AZA and its derivative 5-2′-deoxycytidine (decitabine), zebularine, and guadecitabin, and the latter class includes hydralazine, procaine, and MG98. Among these agents, azacitidine and decitabine have been approved by the US Food and Drug

Administration (FDA) for the treatment of myelodysplastic syndrome [43]. Whether these demethylating agents can be therapeutically beneficial in different disease settings is interesting to confirm. Moreover, ECRG4 is secreted, tethered to the surface, and proteolytically processed for biological activity, thereby offering considerable potential for new drug discovery. Either agonists to its high affinity receptors or protease inhibitors of cell surface processing are interesting targets for drug development.

Conclusions

Twenty years of exploration revealed more and more information on ECRG4. The gene is constitutively expressed on quiescent tissues as a sentinel-like molecule. Upon injury, inflammation, or infection, the expression of *ECRG4* is downregulated to permit the proliferation of injured cells for tissue repair. Then, *ECRG4* expression returns to restore quiescence. The dysregulation of ECRG4 leads to a variety of diseases, including cancer. The anti-tumor effect induces the interplay between leukocytes and tumor cells. In addition, ECRG4 can be easily detected in body fluids, and its presence predicts the clinical outcome in certain diseases. All of these properties illuminate the potential of ECRG4 as a target in precision medicine. However, its clinical significance entails a lengthy confirmation process. First, similar to numerous other biomarkers for cancer, the strong correlation of ECRG4 with cancer progression is mostly based on the "experiment-control" studies by comparing the expression changes between normal tissues and cancer tissues with semiquantitative approaches, whereas the bench-to-bed translation to the clinic requires the confirmation of the data with full-quantitative approaches. Second, additional works on patients are required for evaluating clinical significance. Third, studies involving KO or transgenic mice models are valuable to uncover the roles of ECRG4 *in vivo* considering that current studies are mostly based on *in-vitro* approaches. Finally, development of new drugs that target ECRG4 requires advanced dissection of the processing mechanism and signaling pathway of ECRG4. Further intensive investigation can provide the complete and clear biological image of ECRG4 and confirm whether the gene can be utilized in precision medicine.

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Compliance with ethics guidelines

Xin Qin and Ping Zhang declare that they have no conflict of interest. This manuscript is a review article and does not involve a research protocol that requires approval by the relevant institutional review board or ethics committee.

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