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Cancer and RASSF1A/RASSF1C, the two faces of Janus.

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Abstract:

Although encoded by the same gene, RASSF1A and RASSF1C are, two opposites in carcinogenesis; one is a tumor suppressor that is silenced in a plethora of cancers, while the other is an oncogene that remains unaffected. Here, we aim to understand why and to highlight their respective clinical implications.

Keywords: RASSF1A, RASSF1C, carcinogenesis, cell homeostasis, C1 domain.

Main Text:

The *RASSF1* gene encodes eight distinct proteins (A-H), arising from differential promoter selection and alternative splicing (Figure1A). The best-characterized isoforms, RASSF1A and RASSF1C, are found ubiquitously in normal tissues and share 60% of similar structural characteristics. Although structurally very close, these isoforms are two antagonists during carcinogenesis. Firstly, RASSF1A inactivation, following hypermethylation of the first *RASSF1* promoter, occurs in many human solid cancers [1] (Figure1A), whereas RASSF1C expression, issued from the second promoter (Figure1A), remains unaffected [2] or even overexpressed [3]. Then, unlike RASSF1A, which functions as a tumor suppressor by stimulating apoptosis and reducing proliferation and migration/invasion [1], RASSF1C emerges as an oncogene and represents the exact opposite activities (Figure1B). For example, in a series of elegant studies,

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Reeves et al. have revealed that RASSF1C overexpression supports tumorigenesis of human breast and lung cancer cells, by up-regulation of both specific growth promoting and EMT marker genes and down-regulation of specific pro-apoptotic genes [3-5], where RASSF1A had no effect [3].

Whereas numerous investigations have reported RASSF1A gene silencing as a deleterious event during tumorigenesis [1, 2], only few studies have simultaneously addressed RASSF1C's involvement in the progression of cancer malignancy [3-7]. These open questions remain: Why do cancer cells need to sustain RASSF1C expression? What properties could underpin tumor suppression to only RASSF1A and not to RASSF1C? What are the clinical consequences of this differential expression?

As scaffold proteins, both are characterized by an ataxia telangiectasia mutated (ATM) kinase phosphorylation site, ~~a~~ Ras-GTPase binding (RA) and Salvador-RASSF-Hippo (SARAH) domains, necessary for interaction with multiple partners that allow them to regulate different signaling pathways such as DNA repair, cell cycle, cell death, invasiveness and motility. Moreover, both variants localize with the microtubules and participate in their stabilization and dynamicity. However, the main difference between RASSF1A and RASSF1C lies at the N-terminus: RASSF1A contains a protein kinase C (PKC) conserved region called C1 domain, lacking at RASSF1C, due to the skipping of exons 1α and 2αβ and the transcription of a single 2γ exon [2] (Figure 1A&B).

The absence of a C1 domain in RASSF1C could explain, at least in part, why RASSF1A and not RASSF1C, acts as a tumor suppressor. Indeed, as a major functional consequence, RASSF1C is unable to associate with the death receptors complex [1], which could account for its inhibitory effects on apoptosis [4]. The C1 domain is also responsible for a unique interaction of RASSF1A with scaffold CSK to restrict SRC family kinase (SFKs) activity and maintain epithelial integrity and cell motility. In RASSF1A-depleted cells, RASSF1C gains the ability to promote SFK's activity, which induces phosphorylation and localization of β-catenin and YAP1 to the nucleus, upregulating the transcription of the target genes implicated in proliferation and invasive phenotypes. Besides, SFKs abolish cell-cell contacts and initiate an EMT-like response with an increase in cell motility [6]. Additionally, even though it is unclear if the C1 domain of RASSF1A plays a role in membrane lipid binding similar to how the C1 domain of PKC binds to membrane lipids [2], one might expect that the difference in functionality of RASSF1A and RASSF1C also relates to their different subcellular localizations.

We must not underestimate the specific affinities of the respective isoforms for their partners as a potential explanation. RASSF1A excels for robust homo- and heterodimerization with other members of the RASSF family through amino-terminal segments, where RASSF1C

exhibits a much lesser ability [8]. Interestingly, homodimerization can improve RASSF1A's protein stability compare to RASSF1C, which is known to be a very unstable protein [9]. In addition, heterodimerization can enlarge the propensity of RASSF1A to other signaling pathways compared to RASSF1C. For instance, the interaction of RASSF1A with activated Ras is mainly attributable to its heterodimerization with RASSF5 (Nore1A), while there is little information describing the role of RASSF1C in Ras signaling. We should keep in mind that RASSF1A functions primarily as the main Ras death effector [2]. Therefore, another scenario is that loss of RASSF1A expression not only disconnects oncogenic Ras from pro-death signaling but also favors RAS driven transformation and metastasis. Consistent with this concept, RASSF1C has been shown to localize to nuclei *via* anchoring to Daxx, a Death domain-associated protein, while RASSF1A only links weakly to Daxx [10]. Degradation of Daxx through DNA damage, leads to RASSF1C release into the cytoplasm where it activates the c-Jun N-terminal kinases (JNK) pathway [10].

Furthermore, since both variants contain similar binding domains, RASSF1C could potentially interfere with RASSF1A's interacting molecules through distinct or overlapping functions. For example, even though both isoforms interact with MST1/2 through the SARAH domain [11], RASSF1C could attenuate MST1/2-mediated apoptosis regulated by RASSF1A [3]. Therefore, RASSF1C, and not RASSF1A, interacts and inhibits βTrCP to induce β-Catenin accumulation, which plays a key role in the Wnt signaling pathway, associated with stimulation of proliferation and prevention of apoptosis in various human cancers [12]. Activation of the ERK1/2 pathway [4] and/or reduction of the AMPK phosphorylation as well as p21 and p27 (cell cycle inhibitors) protein levels [5], represent two other possible mechanisms that contribute to cancer progression of RASSF1C-overexpressed cancer cells. RASSF1C stimulates the expression of other interesting genes such as the stem cell self-renewal Piwi-like protein 1 (PIWIL1) that is over-expressed in several human cancers through MEK-ERK1/2 pathway [5]. Lastly, it has been revealed that RASSF1C attenuates miR-33a expression, which is an inhibitor of pro-EMT genes including β-catenin, vimentin and snail [7].

Evidently, predominant expression of either RASSF1A or RASSF1C could explain the ambivalence and duality of the biological functions of the *RASSF1* gene. Depending on the cellular physiological context, each isoforms may be more or less important than the other, and their expression ratio is probably the crucial determinant that influences their activity and the cell's fate. Thus, during tumorigenesis and due to selective suppression of RASSF1A expression, the equilibrium between the RASSF1A and RASSF1C isoforms is disrupted. Consequently, the abnormal protein expression provides opportunity for cancerous cells to take advantage of this imbalance, and promotes growth, survival, and prevention of cell death.

Although RASSF1C's expression is not routinely analyzed, it could also hold clinical value as both a prognostic and predictive biomarker. Indeed, it has been found that overexpression of RASSF1C reduces drug sensitivity [4, 5]. The therapeutic approaches aimed at restoring expression of genes silenced by hypermethylation have yet to be proven effective, partly due to their non-specific action. An alternative could be to counteract the action of RASSF1C in order to fight the cell disorders of the RASSF1A-depleted cancer cells. With this objective, an attractive therapeutic avenue to pursue could be the inhibition of ERK or YAP, which are both increased in RASSF1C-overexpressed cancer cells ([4] and [6], respectively) (Figure2).

Given the known dual role of the *RASSF1* gene, it is important for cancer researchers to consider the balance between RASSF1A and RASSF1C in their experiments in order to gain a better understanding of their distinct and/or overlapping contribution to oncogenesis. More effort is needed to elucidate the actual functions of RASSF1A and RASSF1C isoforms in different cancer types, which is also a major research direction in our future study. Finally, we must not forget that research on the functions of the RASSF1B, D, E, F, G and H isoforms is still in its early days.

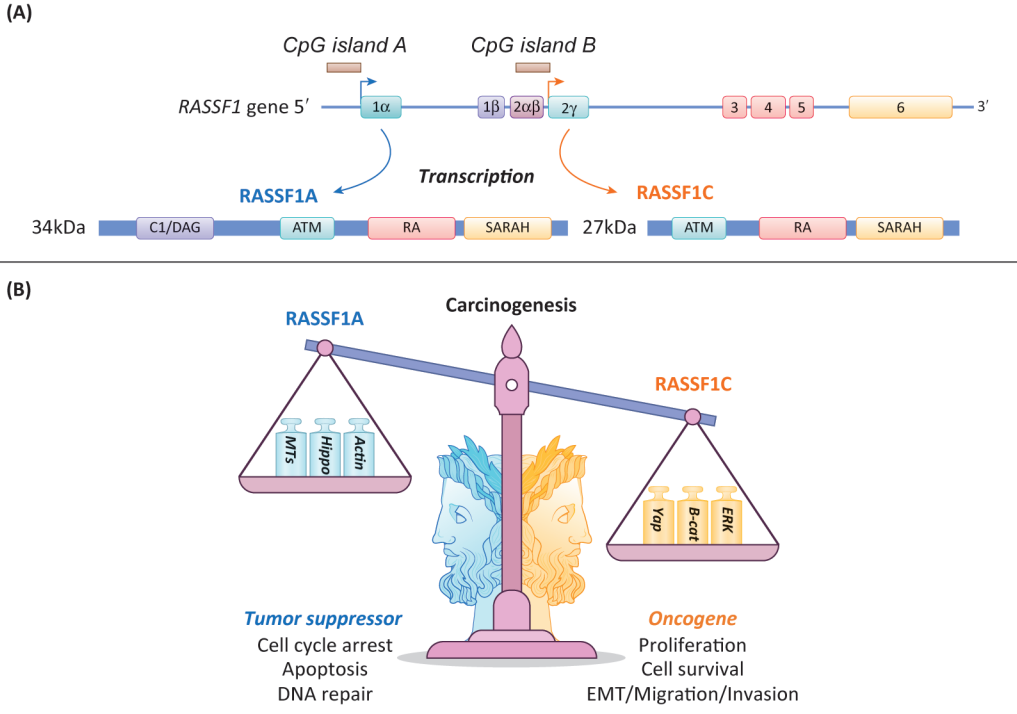
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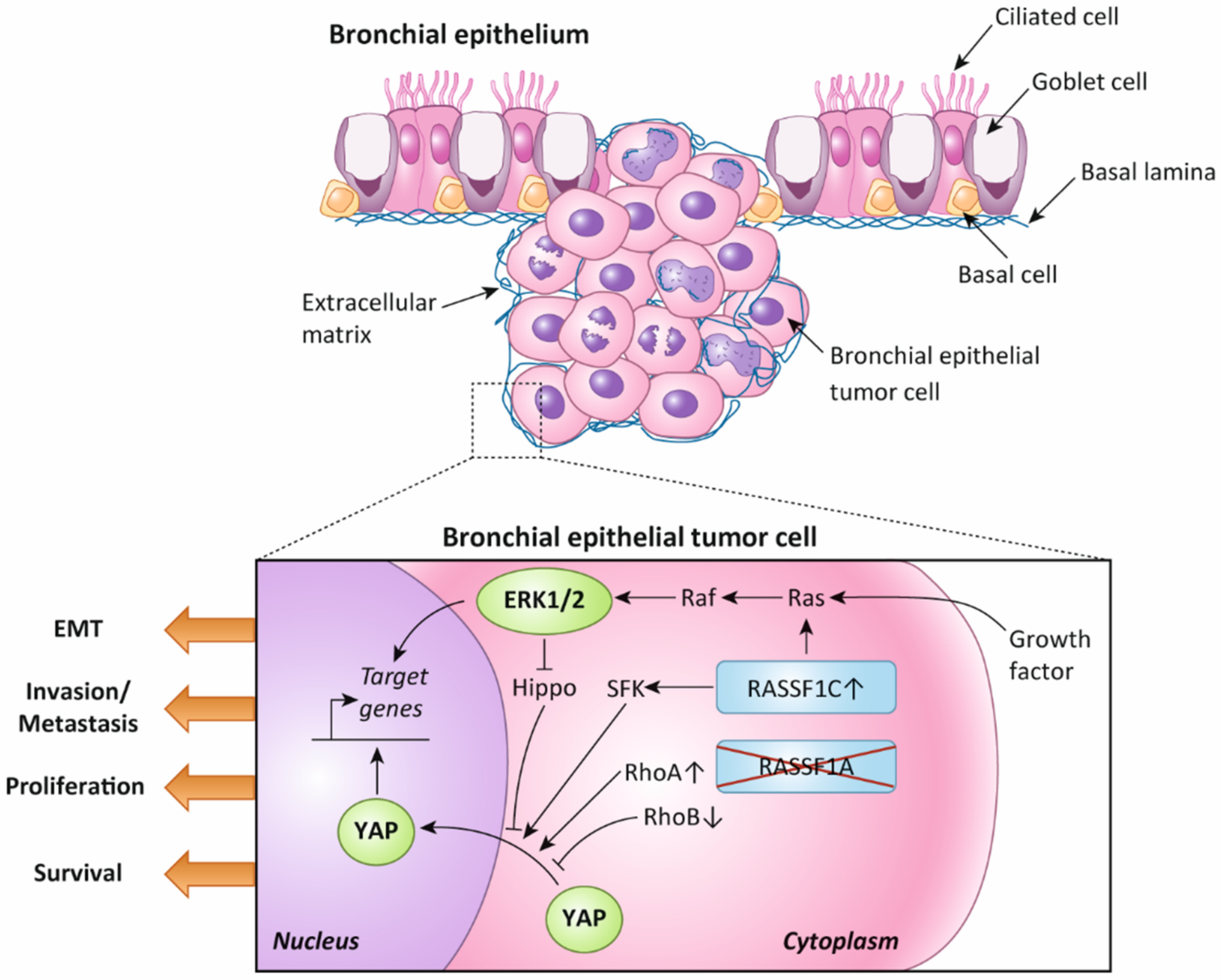
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Figure 1: RASSF1A and RASSF1C, the two faces of Janus during carcinogenesis. Janus, the god of beginnings and ends, choices and duality, is represented with one face turned to the past, the other to the future. We can transpose RASSF1A and RASSF1C to these two faces: RASSF1A faces to the healthy cell while RASSF1C faces to their malignant transformation. **(A)** Structure of *RASSF1* gene, containing two CpG island. Alternative splicing and promoter selection give rise to eight isoforms RASSF1 (A-H). RASSF1A and RASSF1C, the best-characterized proteins, play scaffold activity through multiple protein-protein interaction. **(B)** The balance between tumor suppressor activity of RASSF1A and oncogenic activity of RASSF1C determines the cell's fate. Upon RASSF1A epigenetic inactivation, through hypermethylation of the first promoter during tumorigenesis, RASSF1C expression remains unaffected or even overexpressed. Consequently, the equilibrium between the RASSF1A and RASSF1C isoforms is disrupted which promotes growth, survival and prevents cell death and cell cycle arrest. The ERK pathway and the upregulation of the transcription of the β-catenin and YAP1 target genes are implicated in proliferation and invasive phenotype of RASSF1C expressed cancer cells. RASSF1A maintains cell phenotype mainly by regulating cytoskeleton (microtubules (MTs) as actin filaments) and the kinases from the Hippo pathway.

Figure 2: Schematic representation of the RASSF1C signaling during malignant transformation of RASSF1A-depleted human bronchial epithelial cells. In RASSF1A-depleted cells, RASSF1C promotes SFK's activity, which induces nuclear localization of YAP. On the other hand, RASSF1C initiates signals that stimulate downstream effectors such as Raf/MEK/ERK pathway. These molecules ultimately upregulate the transcription of the target genes implicated in proliferation, EMT and invasive phenotypes. Thus, not only RASSF1A and RASSF1C can be used as potential prognostic and predictive biomarker but also YAP or ERK1/2 pharmacologic targeting in RASSF1A-methylated tumors, could be efficient in preventing metastasis spread, and improve lung cancer overall survival.


(A)
CpG island A
CpG island B
RASSF1 gene 5′
1α
1β
2αβ
2γ
3
4
5
6
3′
Transcription
RASSF1A
RASSF1C
34kDa
C1/DAG
ATM
RA
SARAH
27kDa
ATM
RA
SARAH
(B)
Carcinogenesis
RASSF1A
RASSF1C
MTs
Hippo
Actin
Yap
B-cat
ERK
Tumor suppressor
Cell cycle arrest
Apoptosis
DNA repair
Oncogene
Proliferation
Cell survival
EMT/Migration/Invasion



Key:

Potential prognostic and predictive biomarker for routine analysis of cancer

Potential therapeutic target in RASSF1A-depleted cancer cells