VAV Proteins

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The VAV GEF family has been traditionally linked to protumorigenic actions in cancer. This idea was reinforced by the use of both cancer cell lines and mouse models demonstrating the proactive role of VAV proteins in the development of different types of tumors, such as skin and breast cancer. However, given the presence of structural domains that facilitate the interaction with a large number of protein partners and the particular features of some of the VAV-dependent pathways, it is conceivable that VAV proteins might antagonize cell transformation in certain in vivo contexts.

Keywords: VAV proteins ; RAC1 ; NOTCH1 ; T-ALL ; PTCL

1. Introduction

RHO GTPase pathways are involved in a wide range of cellular processes, such as proliferation, cell survival, and apoptosis, as well as cell-specific roles in many tissues regulating immune response, angiogenesis, and neurogenesis [1]. Thus, alterations of these pathways contribute to a large variety of relevant pathologies, including malignant transformation and cancer progression [1][2]. Historically, it has been assumed that the main mechanism of deregulation of RHO GTPases and their regulators in cancer was through changes in the expression levels [1][3][4]. Although this paradigm remains valid in some tumors, this idea has been challenged with the development of deep sequencing technologies, as some members of the RHO family (RAC1, RHOA, and CDC42) and their regulators (RHO GDP/GTP exchange factors (GEFs), such as P-REX2 and VAV1) [5] have been found mutated in a large number of human tumors [1]. Furthermore, the detection of not only gain-of-function but also loss-of-function mutations in RHO GTPase-related genes underscores the complexity of the RHO GTPase routes in human tumors.

2. VAV1 as a Tumor Suppressor in T-ALLs

Three decades ago, VAV1 was identified as an oncogene by the classical focus formation assays in NIH3T3 cells [6]. This connection with protumorigenic events continued to be supported by accumulative evidence from both cell lines and mouse models [7][8][9][10]. Despite this evidence, and perhaps counter-intuitively, the role of VAV1 in hematological malignancies did not appear to be significant. This view started to change upon the recent discovery of putative gain-of-function mutations in the VAV1 gene in human tumors. Indeed, recent genomic research using whole exome sequencing revealed that 2–17% of peripheral T-cell lymphomas (PTCLs) and 2–3% of lung tumor cases bear VAV1 mutations [11][12][13][14][15][16][17][18]. Given this background, it was quite surprising that VAV1-deficient mice develop T cell tumors at high frequency upon aging or when treated with different carcinogens [19][20]. This phenotype is driven exclusively by VAV1 deficiency, as compound VAV1−/−; VAV2−/−; VAV3−/− animals behave similarly to their VAV1−/− counterparts. The analysis of these tumor-bearing animals revealed the presence of abnormally high numbers of immature T cells in the thymus. Consistent with this, they develop T cell acute lymphoblastic leukemia (T-ALL), a type of cancer that arises from the malignant transformation of immature T lymphocytes lacking T cell receptor (TCR) expression [21]. Furthermore, flow cytometry analysis revealed that VAV1 could act as a tumor suppressor protein at different levels during T cell maturation. This result suggests a hitherto unknown function for VAV1, since it has been traditionally associated with TCR selection and antigen-dependent TCR signaling events in mature T cells [22]. Surprisingly, in silico analysis revealed a high degree of similarity of the VAV1−/− tumor transcriptome with that found deregulated in leukemic cells generated after ectopic expression of the intracellular domain of Notch1 (ICN1) in mouse bone marrow progenitors. In agreement with these data, transcripts commonly upregulated in Notch1-driven T- ALL such as Hes1 and Myc were found in VAV1−/− tumor cells using qRT-PCR experiments. Overall, these data suggest a novel relationship between VAV1 and the NOTCH1 pathway.

The strong oncogenic activity of NOTCH1, which is commonly mutated in more than 65% of human T-ALL cases, has emerged as a major regulator of T-ALL development [21]. NOTCH1 signaling is initiated upon binding of transmembrane ligands expressed on the surface of neighboring cells. Following this interaction, two successive proteolytic cleavages carried out by ADAM10 metalloprotease and γ-secretase complex, respectively, lead to migration of the intracellular

domain (ICN1) to the nucleus and stimulation of its target genes involved in cell fate decision, metabolism and proliferation [21][23]. This transcriptional program ends with phosphorylation of the C-terminal PEST region, which targets ICN1 for FBXW7-mediated ubiquitination and degradation by the proteasome [24]. Most T-ALL-associated mutations result in the truncation of the PEST domain allowing ICN1 to evade proteasomal degradation. Therefore, NOTCH1 signaling is constitutively active in these types of tumors [25]. Our study revealed a defective ubiquitination of ICN1 in the absence of VAV1 in T cells, indicating a connection of VAV1 to the silencing step of NOTCH1 signaling through degradation [19]. Based on these results, we suspected that the best candidate for this regulatory step could be the E3 ubiquitin ligase CBL-B since it can bind to the SH3 C-terminal (CSH3) domain of VAV1[26]. This novel adaptor function mediated by VAV1 through its C-terminal region favors the formation of cytosolic complexes between ICN1 and CBL-B and facilitates the CBL-B–mediated degradation of ICN1 in T cells by the proteasome. As a result, the ablation of VAV1 in mice leads to unbalanced Notch1 signaling, the activation of Notch1 target gene signatures, and the rapid emergence of T-ALL [5][19]. Further experiments demonstrated that the central catalytic core or the SH2 domain of VAV1 are not relevant in the formation of these complexes. Indeed, this new catalytic-independent function is still active in unstimulated T cells, demonstrating that VAV1–ICN1 connection is tyrosine phosphorylation-independent[5][19]. In line with this, we have observed that the engagement of this signaling mechanism does not require, unlike all the other catalysis-dependent and -independent functions so far known for VAV1 [7], a proper activation of the TCR or the prior phosphorylation of the protein, demonstrating that VAV1–ICN1 connection is tyrosine phosphorylation-independent [19]. According to these data, the VAV1–CBL-B suppressor pathway might be active in immature thymocytes (lacking TCR expression) as a way to ensure normal levels of ICN1 signaling during T cell maturation. All together, these results unveiled a new process for the regulation of ICN1 abundance in T lymphocytes that does not relies on its canonical degradation by FBXW7.

Finally, and most importantly, researchers also provide evidence supporting the idea that the downmodulation of this tumor suppressor route is important for the pathogenesis of human T-ALL patients. Among the various T-ALL oncogenic alterations reported to date, gain-of-function alterations in transcriptional factors such as LYL1, HOXA, TAL1, TLX1, and TLX3 represent a recurrent oncogenic hallmark of T-ALL [23]. In silico analyses of patient samples representative of these molecular T-ALL disease subtypes indicated that TLX+ T-ALL clinical subtype cases showed low abundance of VAV1 transcript as well as a similar gene signature to the murine VAV1−/− T-ALL cells [19]. Independent studies have demonstrated that TLX proteins are the most relevant oncogenic drivers for this subtype of T-ALL in humans [21][27]. Moreover, these proteins can directly repress the VAV1 gene, resulting in a downmodulation of the tumor suppressor role of the protein. In line with this, primary cells directly obtained from TLX+ T-ALL patients showed much lower levels of VAV1 protein than patient-derived TLX– T-ALL cells. Consistent with these observations, the re-expression of VAV1 resulted in antiproliferative and pro-apoptotic effects in all the TLX+ cells tested. However, these effects could not be elicited when a VAV1 mutant protein incapable of binding CBL-B was used in the same experiments [19]. In line with the non-catalytical function of VAV1 described above, we also showed that the negative effect of ectopically expressed VAV1 in the fitness of TLX+ T-ALL cells could be recapitulated by the expression of a catalytically dead mutant of the protein unable to bind RHO GTPases. Taken together, these results highlighted the clinical significance of the VAV1–CBL-B suppressor pathway silencing in this leukemia subtype.

3. How Widespread Is This Tumor Suppressor Function in Neoplastic Processes?

As summarized above, this is the first time that the transcriptional repression of VAV1 gene appears to represent a key factor contributing to human cancer pathogenesis [19]. Contrary to this scenario, different cancer studies have reported unexpected expression of VAV1 in tissues where it is not normally expressed, such as pancreas and lung cancer [28][29]. This evidence suggests that the downregulation of the VAV1–CBL-B axis may not be relevant in other cancer types beyond T-ALL. However, the discovery of point mutations as well as gene fusions and truncations targeting the CSH3 domain of VAV1 predicts the importance of the VAV1 tumor suppressor-like pathway [30]. These alterations were found in both T cell neoplasms (mainly PTCLs) and lung tumors. According to these studies, the CSH3 region is the most commonly mutated hotspot of the protein (≈50% of the VAV1 mutations). Therefore, the alterations in the CSH3 domain, which is also responsible for the intramolecular inhibition of the protein[30], suggest an increase in the catalytic-dependent and independent outputs of the protein, as well as the prevention of the CBL-B–ICN1 complex formation favoring the development of human tumors. However, the relevance of these mutations from a functional and pathobiological perspective remains undefined. In this context, recent studies have shown that some VAV1 mutations targeting the CSH3 domain lead indeed to gain-of-function events [11]. However, these analyses have been limited to a small and overlapping subset of mutations targeting obvious regulatory layers of the protein. As a result, we do not know yet whether most VAV1 mutations found in human tumors act as bona fide oncogenic drivers in vivo and, if so, whether they do it autonomously or in combination with other genetic lesions.

In line with this, we decided to analyze the lymphomagenic potential of VAV1 mutations using an adoptive T cell transfer approach (J.R.-V., submitted paper). Our in vivo experiments indicated that a CSH3-terminally truncated protein that leads to the concurrent hyperstimulation of the RAC1 and NFAT pathways as well as the NOTCH1-derived signals is able to autonomously drive PTCL-like lymphomas in mice, specifically angioimmunoblastic T-cell lymphoma (AITL) subtype (J.R.-V., submitted paper). These animals exhibited a highly expanded population of CD4 + T cells with a T follicular helper (T FH) cell-like phenotype, a common feature found in previously reported AITL-associated cases in both mice and humans [31]. The contribution of VAV1 mutations in the development of T cell neoplasms in mice comes as no surprise. In a recent paper, it has been reported that transgenic mice expressing other oncogenic versions of VAV1 can develop GATA3 + PTCL-not otherwise specified–like (PTCL-NOS) tumors in the presence of Trp53 deletion [32]. The lack of detection of this PTLC subtype in our adoptive transfer experiments is still unclear, although it might reflect the need of cooperating genetic events that could favor the emergence of a T cell subtype different from the T FH phenotype characteristic of AITL (e.g., viral integrations in the case of adult T cell leukemia/lymphoma [ATLL], loss of TP53 and/or TET2 genes in AITL) [31][32][33]. The use of animal model-based experiments will help to understand the etiology of PTCL subtypes in the near future.

VAV CSH3 domain also facilitates the interaction with a large number of proteins such as the heterogenous nuclear ribonucleoprotein K (HNRNPK) and dynamin 2 (DNM2)[34][35][36]. We cannot exclude the possibility that some mutations preferentially prevent the binding of either HNRNPK or DNM2. The functional implication of this putative change in the spectrum of binding partners is currently unknown. However, it is worth noting that, similarly to CBL-B, HNRNPK and some DNM family members (e.g., DNM3) have been associated to tumor suppression activities [36][37]. Taken together, these proteins might play further adaptor-like functions in both normal and cancer cells, expanding the catalogue of VAV-dependent suppressor activities.

References

1. Bustelo, X.R. RHO GTPases in cancer: Known facts, open questions, and therapeutic challenges. Biochem. Soc. Trans. 2018, 46, 741–760.
2. Porter, A.P.; Papaioannou, A.; Malliri, A. Deregulation of Rho GTPases in cancer. Small GTPases 2016, 7, 123–138.
3. Cook, D.R.; Rossman, K.L.; Der, C.J. Rho guanine nucleotide exchange factors: Regulators of Rho GTPase activity in development and disease. Oncogene 2014, 33, 4021–4035.
4. Sahai, E.; Marshall, C.J. RHO-GTPases and cancer. Nat. Rev. Cancer 2002, 2, 133–142.
5. Robles-Valero, J.; Lorenzo-Martín, L.F.; Fernández-Pisonero, I.; Bustelo, X.R. Rho guanosine nucleotide exchange factors are not such bad guys after all in cancer. Small GTPases 2020, 11, 233–239.
6. Katzav, S.; Martin-Zanca, D.; Barbacid, M. vav, a novel human oncogene derived from a locus ubiquitously expressed in hematopoietic cells. EMBO J. 1989, 8, 2283–2290.
7. Bustelo, X.R. Vav family exchange factors: An integrated regulatory and functional view. Small GTPases 2014, 5, 9.
8. Citterio, C.; Menacho-Márquez, M.; García-Escudero, R.; Larive, R.M.; Barreiro, O.; Sánchez-Madrid, F.; Paramio, J.M.; Bustelo, X.R. The rho exchange factors vav2 and vav3 control a lung metastasis-specific transcriptional program in breast cancer cells. Sci. Signal. 2012, 5, ra71.
9. Menacho-Márquez, M.; García-Escudero, R.; Ojeda, V.; Abad, A.; Delgado, P.; Costa, C.; Ruiz, S.; Alarcón, B.; Paramio, J.M.; Bustelo, X.R. The Rho exchange factors Vav2 and Vav3 favor skin tumor initiation and promotion by engaging extracellular signaling loops. PLoS Biol. 2013, 11, e1001615.
10. Chang, K.H.; Sanchez-Aguilera, A.; Shen, S.; Sengupta, A.; Madhu, M.N.; Ficker, A.M.; Dunn, S.K.; Kuenzi, A.M.; Arnett, J.L.; Santho, R.A.; et al. Vav3 collaborates with p190-BCR-ABL in lymphoid progenitor leukemogenesis, proliferation, and survival. Blood 2012, 120, 800–811.
11. Abate, F.; da Silva-Almeida, A.C.; Zairis, S.; Robles-Valero, J.; Couronne, L.; Khiabanian, H.; Quinn, S.A.; Kim, M.Y.; Laginestra, M.A.; Kim, C.; et al. Activating mutations and translocations in the guanine exchange factor VAV1 in peripheral T-cell lymphomas. Proc. Natl. Acad. Sci. USA 2017, 114, 764–769.
12. Crescenzo, R.; Abate, F.; Lasorsa, E.; Tabbo', F.; Gaudiano, M.; Chiesa, N.; Di Giacomo, F.; Spaccarotella, E.; Barbarossa, L.; Ercole, E.; et al. Convergent mutations and kinase fusions lead to oncogenic STAT3 activation in anaplastic large cell lymphoma. Cancer Cell 2015, 27, 516–532.
13. Boddicker, R.L.; Razidlo, G.L.; Dasari, S.; Zeng, Y.; Hu, G.; Knudson, R.A.; Greipp, P.T.; Davila, J.I.; Johnson, S.H.; Porcher, J.C.; et al. Integrated mate-pair and RNA sequencing identifies novel, targetable gene fusions in peripheral T-cell lymphoma. Blood 2016, 128, 1234–1245.

14. Fujisawa, M.; Sakata-Yanagimoto, M.; Nishizawa, S.; Komori, D.; Gershon, P.; Kiryu, M.; Tanzima, S.; Fukumoto, K.; Enami, T.; Muratani, M.; et al. Activation of RHOA-VAV1 signaling in angioimmunoblastic T-cell lymphoma. Leukemia 2018, 32, 694–702.

15. Kataoka, K.; Nagata, Y.; Kitanaka, A.; Shiraishi, Y.; Shimamura, T.; Yasunaga, J.; Totoki, Y.; Chiba, K.; Sato-Otsubo, A.; Nagae, G.; et al. Integrated molecular analysis of adult T cell leukemia/lymphoma. Nat. Genet. 2015, 47, 1304–1315.

16. Park, J.; Yang, J.; Wenzel, A.T.; Ramachandran, A.; Lee, W.J.; Daniels, J.C.; Kim, J.; Martinez-Escala, E.; Amankulor, N.; Pro, B.; et al. Genomic analysis of 220 CTCLs identifies a novel recurrent gain-of-function alteration in RLTPR (p.Q575E). Blood 2017, 130, 1430–1440.

17. Vallois, D.; Dobay, M.P.; Morin, R.D.; Lemonnier, F.; Missiaglia, E.; Juilland, M.; Iwaszkiewicz, J.; Fataccioli, V.; Bisig, B.; Roberti, A.; et al. Activating mutations in genes related to TCR signaling in angioimmunoblastic and other follicular helper T-cell-derived lymphomas. Blood 2016, 128, 1490–1502.

18. Yoo, H.Y.; Sung, M.K.; Lee, S.H.; Kim, S.; Lee, H.; Park, S.; Kim, S.C.; Lee, B.; Rho, K.; Lee, J.E.; et al. A recurrent inactivating mutation in RHOA GTPase in angioimmunoblastic T cell lymphoma. Nat. Genet. 2014, 46, 371–375.

19. Robles-Valero, J.; Lorenzo-Martín, L.F.; Menacho-Márquez, M.; Fernández-Pisonero, I.; Abad, A.; Camós, M.; Toribio, M.L.; Espinosa, L.; Bigas, A.; Bustelo, X.R. A Paradoxical Tumor-Suppressor Role for the Rac1 Exchange Factor Vav1 in T Cell Acute Lymphoblastic Leukemia. Cancer Cell 2017, 32, 608–623.e609.

20. Ruiz, S.; Santos, E.; Bustelo, X.R. The use of knockout mice reveals a synergistic role of the Vav1 and Rasgrf2 gene deficiencies in lymphomagenesis and metastasis. PLoS ONE 2009, 4, e8229.

21. Van Vlierberghe, P.; Ferrando, A. The molecular basis of T cell acute lymphoblastic leukemia. J. Clin. Investig. 2012, 122, 3398–3406.

22. Fujikawa, K.; Miletic, A.V.; Alt, F.W.; Faccio, R.; Brown, T.; Hoog, J.; Fredericks, J.; Nishi, S.; Mildiner, S.; Moores, S.L.; et al. Vav1/2/3-null mice define an essential role for Vav family proteins in lymphocyte development and activation but a differential requirement in MAPK signaling in T and B cells. J. Exp. Med. 2003, 198, 1595–1608.

23. Andersson, E.R.; Sandberg, R.; Lendahl, U. Notch signaling: Simplicity in design, versatility in function. Development 2011, 138, 3593–3612.

24. O'Neil, J.; Grim, J.; Strack, P.; Rao, S.; Tibbitts, D.; Winter, C.; Hardwick, J.; Welcker, M.; Meijerink, J.P.; Pieters, R.; et al. FBW7 mutations in leukemic cells mediate NOTCH pathway activation and resistance to gamma-secretase inhibitors. J. Exp. Med. 2007, 204, 1813–1824.

25. Weng, A.P.; Ferrando, A.A.; Lee, W.; Morris, J.P.; Silverman, L.B.; Sanchez-Irizarry, C.; Blacklow, S.C.; Look, A.T.; Aster, J.C. Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. Science 2004, 306, 269–271.

26. Bustelo, X.R.; Crespo, P.; Lopez-Barahona, M.; Gutkind, J.S.; Barbacid, M. Cbl-b, a member of the Sli-1/c-Cbl protein family, inhibits Vav-mediated c-Jun N-terminal kinase activation. Oncogene 1997, 15, 2511–2520.

27. Della Gatta, G.; Palomero, T.; Perez-Garcia, A.; Ambesi-Impiombato, A.; Bansal, M.; Carpenter, Z.W.; De Keersmaecker, K.; Sole, X.; Xu, L.; Paietta, E.; et al. Reverse engineering of TLX oncogenic transcriptional networks identifies RUNX1 as tumor suppressor in T-ALL. Nat. Med. 2012, 18, 436–440.

28. Fernandez-Zapico, M.E.; Gonzalez-Paz, N.C.; Weiss, E.; Savoy, D.N.; Molina, J.R.; Fonseca, R.; Smyrk, T.C.; Chari, S.T.; Urrutia, R.; Billadeau, D.D. Ectopic expression of VAV1 reveals an unexpected role in pancreatic cancer tumorigenesis. Cancer Cell 2005, 7, 39–49.

29. Lazer, G.; Idelchuk, Y.; Schapira, V.; Pikarsky, E.; Katzav, S. The haematopoietic specific signal transducer Vav1 is aberrantly expressed in lung cancer and plays a role in tumourigenesis. J. Pathol. 2009, 219, 25–34.

30. Barreira, M.; Fabbiano, S.; Couceiro, J.R.; Torreira, E.; Martinez-Torrecuadrada, J.L.; Montoya, G.; Llorca, O.; Bustelo, X.R. The C-Terminal SH3 Domain Contributes to the Intramolecular Inhibition of Vav Family Proteins. Sci. Signal. 2014, 7, ra35.

31. Zang, S.; Li, J.; Yang, H.; Zeng, H.; Han, W.; Zhang, J.; Lee, M.; Moczygemba, M.; Isgandarova, S.; Yang, Y.; et al. Mutations in 5-methylcytosine oxidase TET2 and RhoA cooperatively disrupt T cell homeostasis. J. Clin. Investig. 2017, 127, 2998–3012.

32. Fukumoto, K.; Sakata-Yanagimoto, M.; Fujisawa, M.; Sakamoto, T.; Miyoshi, H.; Suehara, Y.; Nguyen, T.B.; Suma, S.; Yanagimoto, S.; Shiraishi, Y.; et al. VAV1 mutations contribute to development of T-cell neoplasms in mice. Blood 2020, 136, 3018–3032.

33. Fiore, D.; Cappelli, L.V.; Broccoli, A.; Zinzani, P.L.; Chan, W.C.; Inghirami, G. Peripheral T cell lymphomas: From the bench to the clinic. Nat. Rev. Cancer 2020, 20, 323–342.

34. Bustelo, X.R.; Suen, K.L.; Michael, W.M.; Dreyfuss, G.; Barbacid, M. Association of the vav proto-oncogene product with poly(rC)-specific RNA-binding proteins. Mol. Cell Biol. 1995, 15, 1324–1332.
35. Gomez, T.S.; Hamann, M.J.; McCarney, S.; Savoy, D.N.; Lubking, C.M.; Heldebrant, M.P.; Labno, C.M.; McKean, D.J.; McNiven, M.A.; Burkhardt, J.K.; et al. Dynamin 2 regulates T cell activation by controlling actin polymerization at the immunological synapse. Nat. Immunol. 2005, 6, 261–270.
36. Gallardo, M.; Lee, H.J.; Zhang, X.; Bueso-Ramos, C.; Pageon, L.R.; McArthur, M.; Multani, A.; Nazha, A.; Manshouri, T.; Parker-Thornburg, J.; et al. hnRNP K Is a Haploinsufficient Tumor Suppressor that Regulates Proliferation and Differentiation Programs in Hematologic Malignancies. Cancer Cell 2015, 28, 486–499.
37. Zhang, Z.; Chen, C.; Guo, W.; Zheng, S.; Sun, Z.; Geng, X. DNM3 Attenuates Hepatocellular Carcinoma Growth by Activating P53. Med. Sci. Monit. 2016, 22, 197–205.