Desenmarañando la progresión de la LLC en pacientes no mutados: relación entre la expresión funcional de la citidina deaminasa inducida por activación con la evolución de la enfermedad

Unraveling CLL progression in unmutated patients: linking functional AID expression with disease evolution

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Activation-induced cytidine deaminase (AID) initiates somatic hypermutation (SHM) and class switch recombination (CSR) of the immunoglobulin (Ig) genes, which are critically important for an effective immune response development[7]. In addition, AID seems to contribute to B cell tolerance in mice and humans eliminating developing autoreactive B cells[5]. As a trade-off of the beneficial roles of AID physiological activity, this enzyme can also contribute to cellular transformation and tumor progression through its off-target mutagenic activity[6]. Mounting evidences indicate a role for AID in disease progression and poor prognosis of chronic lymphoid neoplasms[8,12]. Since these leukemias are not immediately derived from germinal center B cells, normal AID regulation might not be fully functional. Specifically, in CLL we have shown that AID is anomalously expressed in the peripheral blood of patients with unmutated (Um) VH genes, correlating with active CSR and poor clinical outcome[9]. In addition, others and us have described that AID expression is mainly restricted to distinct proliferative subsets within the leukemic clone[1,10,11]. Despite the fact that AID expression has been associated with adverse cytogenetics and worse outcome[4] definitive experimental demonstration that AID contributes to CLL progression is missing.

To obtain insight into this issue we analyzed mutation load and signatures in Um patients in which anomalous expression of AID in peripheral blood was sustained throughout disease evolution. We compared the mutational profile by Whole Exome

Sequencing (WES) of purified CD19+ cells extracted from 5 progressive patients expressing AID (Um/AID^{pos}) and from 5 progressive patients in which AID was not detected (Um/AID^{neg}). Samples were obtained at diagnosis and at second time point after the first treatment (AFT). Analysis was performed according to GATK-best practice guidelines, variant discovery was performed by Varscan algorithm. Unsupervised AID mutational profile was assessed by the Bayesian nonnegative matrix factorization algorithm as previously described[2], with this strategy we identified non-canonical AID signatures (nc-AID), i.e. A>C mutations at WA motifs in sense strand and T>G mutation at TW motifs in antisense strand. For supervised analysis of canonical AID signatures (c-AID) we quantified C to T mutations at WRCY motifs in the sense strand or T to C variants in YGRW motifs in antisense strand.

We found that the total number of somatic mutations is significantly increased in Um/AID^{pos} compared with Um/AID^{neg} patients independently of the disease time of the analyzed sample. A higher frequency of somatic mutations were observed in Um/AID^{pos} samples at debut of the disease compared with Um/AID^{pos} samples AFT. We next analyzed mutation signatures in the different groups: Um/AID^{pos} at diagnosis; Um/AID^{pos} AFT; Um/AID^{neg} at diagnosis and Um/AID^{neg} AFT. Our results show no significant differences observed between the groups concerning c-AID mutations. However, an increased and significant difference was found in the group Um/AID^{pos} compared with Um/AID^{neg}. Interestingly, a higher frequency of nc-AID mutations was observed in Um/AID^{pos} group at diagnosis time compared with Um/AID^{pos} AFT, which might suggest selection for samples with lower AID levels and/or mutation loads. In addition, the unsupervised analysis identified the presence other previously described signatures[2,3], such as Aging, and APOBEC in all samples. We also analyzed AID-catalyzed mutations that were found at the debut of the disease and maintained during disease evolution, as well as those that appeared only in the AFT samples. Presently, mutations in selected genes are being confirmed in a larger cohort. We will discuss these results.

AID expression could be especially problematic in chronic diseases, where even a small but continuous level of AID activity can lead to selectable genetic mutations over time, giving rise to more aggressive tumors and treatment resistance. Our results support this notion and describe for the first time the mutational profile of Um and progressive patients with anomalous AID expression through their evolution. Under the light of the hypothesis that AID expression is a hallmark of an activated microenvironment resulting in disease progression our results will allow us to identify AID mutated genes which could be implicated in CLL tumor progression.

References

1. Albesiano, E., Messmer, B. T., Damle, R. N., Allen, S. L., Rai, K. R., and Chiorazzi, N. Activation-induced cytidine deaminase in chronic lymphocytic leukemia B cells: expression as multiple forms in a dynamic, variably sized fraction of the clone. Blood. 2003;*102*, 3333-3339.
2. Alexandrov, L. B., Nik-Zainal, S., Wedge, D. C., Aparicio, S. A., Behjati, S., Biankin, A. V., Bignell, G. R., Bolli, N., Borg, A., Borresen-Dale, A. L. *et al.* Signatures of mutational processes in human cancer. Nature . 2013;*500*, 415-421.
3. Kasar, S., Kim, J., Improgo, R., Tiao, G., Polak, P., Haradhvala, N., Lawrence, M. S., Kiezun, A., Fernandes, S. M., Bahl, S. *et al.* Whole-genome sequencing reveals activation-induced cytidine deaminase signatures during indolent chronic lymphocytic leukaemia evolution. Nat Commun. 2015;*6*, 8866.
4. Leuenberger, M., Frigerio, S., Wild, P. J., Noetzli, F., Korol, D., Zimmermann, D. R., Gengler, C., Probst-Hensch, N. M., Moch, H., and Tinguely, M. AID protein expression in chronic lymphocytic leukemia/small lymphocytic lymphoma is associated with poor prognosis and complex genetic alterations. Mod Pathol. 2010;*23*, 177-186.
5. Meyers, G., Ng, Y. S., Bannock, J. M., Lavoie, A., Walter, J. E., Notarangelo, L. D., Kilic, S. S., Aksu, G., Debre, M., Rieux-Laucat, F. *et al.* Activation-induced cytidine deaminase. AID) is required for B-cell tolerance in humans. Proceedings of the National Academy of Sciences of the United States of America. 2011;*108*, 11554-11559.

6. Montamat-Sicotte D., Palacios F., and, D.-N. J., and Oppezzo, P. Origins and consequences of AID expression in lymphoid neoplasms. Current Immunology Reviews. 2013;*9*, 75-85.

7. Muramatsu, M., Kinoshita, K., Fagarasan, S., Yamada, S., Shinkai, Y., and Honjo, T. Class switch recombination and hypermutation require activation-induced cytidine deaminase. AID), a potential RNA editing enzyme. Cell. 2000;*102*, 553-563.

8. Okazaki, I. M., Hiai, H., Kakazu, N., Yamada, S., Muramatsu, M., Kinoshita, K., and Honjo, T. Constitutive expression of AID leads to tumorigenesis. J Exp Med. 2003;*197*, 1173-1181.

9. Oppezzo, P., Vuillier, F., Vasconcelos, Y., Dumas, G., Magnac, C., Payelle-Brogard, B., Pritsch, O., and Dighiero, G. Chronic lymphocytic leukemia B cells expressing AID display a dissociation between class switch recombination and somatic hypermutation. Blood. 2003 May 15;101(10):4029-32.

10. Palacios, F., Moreno, P., Morande, P., Abreu, C., Correa, A., Porro, V., Landoni, A. I., Gabus, R., Giordano, M., Dighiero, G. *et al.* High expression of AID and active class switch recombination might account for a more aggressive disease in unmutated CLL patients: link with an activated microenvironment in CLL disease. Blood. 2010;*115*, 4488-4496.

11. Patten, P. E., Chu, C. C., Albesiano, E., Damle, R. N., Yan, X. J., Kim, D., Zhang, L., Magli, A. R., Barrientos, J., Kolitz, J. E. *et al.* IGHV unmutated and mutated chronic lymphocytic leukemia cells produce activation-induced deaminase protein with a full range of biologic functions. Blood. 2012 Dec 6;120(24):4802-11.

12. Robbiani, D. F., Bunting, S., Feldhahn, N., Bothmer, A., Camps, J., Deroubaix, S., McBride, K. M., Klein, I. A., Stone, G., Eisenreich, T. R. *et al.* . AID produces DNA double-strand breaks in non-Ig genes and mature B cell lymphomas with reciprocal chromosome translocations. Mol Cell. 2009)*36*, 631-641.