APOBEC3B spreads somatic mutations in breast cancer genome

Giusi Irma Forte

IBFM CNR - LATO, Contrada Pietrapollastra Pisciotto, 90015, Cefalù (PA), Italy
Corresponding to: Giusi Irma Forte, PhD. IBFM CNR - LATO, Contrada Pietrapollastra Pisciotto, 90015, Cefalù (PA), Italy. Email: giusi.forte@ibfm.cnr.it.

Cancer progression is characterized by a well-known step-wise somatic mutation accumulation mechanism, by which tumorigenic cells acquire a gradually more malign phenotype and override cell death signals.

During our genomic era, the landscape of somatic mutations has been largely studied in several types of carcinomas, producing evidences that the mutation spectra can be different at individual and clonal level, though some of these modifications, as well as, some chromosomal alterations could be repeatedly observed in similar types of tumours (1-4). Moreover, flow-sorted tumour cell clones, separated according DNA index and analysed for their genome-wide allelic state, show a gain in somatic mutations and chromosomal alterations in those cell fractions having a higher H-index, even if the clones share common alterations previously inserted (5). Then, the mutation accumulation on focused targets, such as oncogenes or cyclins, as well as, the unfocused genome allelic state changes are signs of disease progression and correlate with a gradually worse prognosis.

However, despite the particular importance of this topic to highlight tumour-progression mechanisms, few information have been collected till now on key factors implicated in cancer somatic mutation spreading.

A decade ago, a protein family composed of the 11 polynucleotide cytosine deaminases: APOBEC1, activation-induced deaminase (AID), APOBEC2, APOBEC3 proteins (A3A, A3B, A3C, A3D, A3F, A3G and A3H) and APOBEC4, having functional capacity to induce mutations in DNA and RNA of living cells, have been discovered (6). These genes are derived from a complex series of duplication and fusion across evolution (6), each one having different and roughly specific targets from cytoplasm to nucleus. Deaminating cytosine to uridine, the APOBEC family members exert a series of fundamental physiological positive activities into cells; some of which are well characterized: APOBEC1 is known to edit the ApoB pre-mRNA (7), whereas the AID conducts an antigen-driven diversification of already rearranged immunoglobulin by somatic hypermutation, gene conversion and Ig class-switch recombination (8). Less is known about APOBEC2 and APOBEC4, which do not seem to display functional activity, instead the APOBEC3 proteins exert a more pronounced retroviral replication inhibition capacity (9), noteworthy is the APOBEC3G role in HIV restriction (10). Such important deaminase family tasks, protecting cells against retroviral attack and enhancing the Ig-molecules ability to recognize a higher number of epitopes, have a dark side of the medal, concerning their role in cancer initiation and evolution, due to a non-controlled C-to-U modification spreading across the genome (11).

In breast cancer, the APOBEC3B (A3B) protein has been recently characterized as a certain enzymatic source of C-to-T mutation dissemination into the genome (12).

Among the seven APOBEC3 members analysed, the A3B mRNA was the only one highly expressed with a fold change ≥3 in 28 out 38 breast cancer cell lines and a fold change ≥10 in 12 out 38 cell lines assayed. Among these, MDA-MB-453, MDA-MB-468 and HCC1569 showed the higher up-regulation, corresponding to fold changes of 20, 21 and 61, respectively. The increased expression levels are hypothesized to be due to such transduction event, as no CpG island modifications or copy number variations have been reported for this gene.

Importantly, during cell cycle the APOBEC3 proteins have different subcellular localizations: cell-wide, cytoplasmic or nuclear, implying that only a subset of APOBEC3s contacts nuclear DNA (13). In the case of MDA-MB-453, MDA-MB-468 and HCC1569 cell lines, a nuclear localization has been observed for an A3B

fluorescent fusion protein. Furthermore, by using a DNA C-to-U fluorescent based assay, a consistent DNA editing has been demonstrated, predominantly regarding cytosine of TC dinucleotides, similarly to a retroviral hypermutation signature caused by A3B overexpression (14).

To address the dimension of the endogenous A3B contribute in DNA C-to-U modification, the genomic uracil load of MDA-MB-453 and HCC1569 has been quantified in comparison to that observed after transfection with an A3B knockdown system, finding an uracil load reduction of 70% and 30%, respectively, in transfected with respect to non-transfected cells. From this observation it has been calculated that, approximately, 30,000 to 60,000 uracils are inserted by A3B per haploid genome.

A corroboration has been obtained measuring the mutation accumulation in engineered MDA-MB-453 and HCC1569 cells expressing the herpes simplex virus type 1 TK gene, which makes the cells sensitive to ganciclovir, then transfecting them with an A3B knockdown system or a control construct. Expanded sub-clones were subjected to ganciclovir selection and resistant cells were grown to form visible colonies, showing that cells with upregulated A3B accumulate 3-5-fold more mutations.

In addition, it has been studied if the mutation accumulation can be considered a targeted or a genome-wide mechanism. In cells highly expressing A3B, TP53 and c-MYC appeared more mutated than CDKN2B, suggesting that such genomic regions are preferentially susceptible to the enzymatic deamination. Other base substitution mutations were rare.

Other effects of the A3B expression have been characterized in engineered Human Embryonic Kidney (HEK) 293 cells, stably expressing A3B. In these cells, besides C-to-T mutations, delayed cell-cycle arrest, abnormal anucleate and multinucleate cell formation, c-H2AX focus formation, DNA fragmentation and eventual cell death have been revealed.

Finally, similar results to those described in cell lines have been searched in primary breast tumours.

A confirmation of the A3B exclusive role in breast cancer cells derive from the observation that only the A3B protein, among the other APOBEC3 members tested, is found upregulated by ≥3 fold in 20 out of 52 primary tumours compared to matched normal tissue and in 44 out of 52 tumours compared to the reduction mammoplasty tissue.

Then, the availability of RNA sequencing (RNA-seq) and somatic mutation databases allowed making a comparison of *in-vivo* and *in-vitro* A3B signatures. This analysis revealed that, whereas C-to-T frequency is low (20%) and random in liver tumour, it is high (80%) in melanoma, focused at dipyrimidines, as expected due to ultraviolet load. Instead, breast cancer is featured by an intermediate (40%) C-to-T frequency, preferentially focused at trinucleotide sites, miming the *in-vitro* A3B signature. Also, the same approach allowed to observe that the A3B upregulation is strongly correlated to mutation accumulation and TP53 inactivation in breast primary tumours and cancer cell lines, being perhaps the TP53 inactivation crucial to override DNA damage stop signals triggered by A3B.

According to the literature, the cytosine deaminase involvement in cancer mutation accumulation and genomic instability is a still not deeply explored argument, as a relative limited number of studies have been dedicated to this topic (14-17), nonetheless their involvement in cancer development is more certain than just supposed.

In this respect, the APOBEC members could represent key factors for targeted therapies, to block mechanisms producing genome-wide alterations, which drive tumour development.

The study described herein makes evident the necessity of future scientific efforts to deeply highlight the cytosine deaminase family contribution and that one of similar molecules in specific tumour types, the causes of their activation and the correlation with grading and prognosis.

Acknowledgments

Footnote

Conflicts of Interest: The author has completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.3978/j.issn.2218-676X.2013.04.03). The author has no conflicts of interest to declare.

Ethical Statement: The author is accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Open Access Statement: This is an Open Access article distributed in accordance with the Creative Commons

References

1. Middeldorp A, van Eijk R, Oosting J, et al. Increased frequency of 20q gain and copy-neutral loss of heterozygosity in mismatch repair proficient familial colorectal carcinomas. Int J Cancer 2012;130:837-46.

2. Gray J, Druker B. Genomics: the breast cancer landscape. Nature 2012;486:328-9.

3. Bravatà V, Cammarata FP, Forte GI, et al. "Omics" of HER2-Positive Breast Cancer. OMICS 2013;17:119-29.

4. Cancer Genome Atlas Research Network. Comprehensive genomic characterization of squamous cell lung cancers. Nature 2012;489:519-25.

5. Corver WE, Middeldorp A, ter Haar NT, et al. Genome wide allelic state analysis on flow-sorted tumor fractions provides an accurate measure of chromosomal aberrations. Cancer Res 2008;68:10333-40.

6. Conticello SG. The AID/APOBEC family of nucleic acid mutators. Genome Biol 2008;9:229.

7. Blanc V, Davidson NO. APOBEC-1-mediated RNA editing. Wiley Interdiscip Rev Syst Biol Med 2010;2:594-602.

8. Pavri R, Nussenzweig MC. AID targeting in antibody diversity. Adv Immunol 2011;110:1-26.

9. Izumi T, Shirakawa K, Takaori-Kondo A. Cytidine deaminases as a weapon against retroviruses and a new target for antiviral therapy. Mini Rev Med Chem 2008;8:231-8.

10. Monajemi M, Woodworth CF, Benkaroun J, et al. Emerging complexities of APOBEC3G action on immunity and viral fitness during HIV infection and treatment. Retrovirology 2012;9:35.

11. Schmitz KM, Petersen-Mahrt SK. AIDing the immune system-DIAbolic in cancer. Semin Immunol 2012;24:241-5.

12. Burns MB, Lackey L, Carpenter MA, et al. APOBEC3B is an enzymatic source of mutation in breast cancer. Nature 2013;494:366-70.

13. Lackey L, Law EK, Brown WL, et al. Subcellular localization of the APOBEC3 proteins during mitosis and implications for genomic DNA deamination. Cell Cycle 2013;12:762-72.

14. Barlow JH, Faryabi RB, Callén E, et al. Identification of early replicating fragile sites that contribute to genome instability. Cell 2013;152:620-32.

15. Ebrahem Q, Mahfouz RZ, Ng KP, et al. High cytidine deaminase expression in the liver provides sanctuary for cancer cells from decitabine treatment effects. Oncotarget 2012;3:1137-45.

16. Andersen A, Jones DA. APC and DNA demethylation in cell fate specification and intestinal cancer. Adv Exp Med Biol 2013;754:167-77.

17. Nik-Zainal S, Alexandrov LB, Wedge DC, et al. Mutational processes molding the genomes of 21 breast cancers. Cell 2012;149:979-93.