Recent insights emerging from malignant mesothelioma genome sequencing

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In this issue of JTO, Lo Iacono *et al.* present a retrospective study performed on 123 formalin-fixed, paraffin-embedded (FFPE) biopsies from 123 malignant mesothelioma (MM) patients, using next generation sequencing (NGS) with a commercially available library of genes that are frequently mutated in cancers. Biopsies were sequenced with the Ion Torrent platform and positive results were validated by Sanger sequencing[1]. Two limitations of this approach, acknowledged by the Authors, are: 1) Since *BAP1* and *NF2* were not represented in the commercial library, their mutations status was analyzed using a different custom-made library; 2) the particular targeted NGS methodology chosen in this paper specifically detects small gene mutations, while this methodology cannot detect large genetic losses, gene amplifications and translocations, or epigenetic changes.

In a parallel study recently published in Cancer Research[2], Guo *et al.* performed whole exome sequencing on DNA and matched blood samples of 22 MM frozen biopsies from 22 patients collected in the operating room, integrated with the analysis of somatic copy number alterations (SCNAs). Exome capture libraries were sequenced on the Illumina HiSeq platform[2]. In both studies the biopsies were from patients previously treated with chemotherapy (100% of patients Lo Iacono *et al.*; 41% Guo *et al.*). Therefore, the well-documented mutagenic effects of chemotherapy on the cancer genome[3] may have contributed to some of the mutations detected. These manuscripts[1, 2] present the first landscape view of the somatic genomic alterations in MM. Because the researchers[1, 2] used different experimental approaches, an exact comparison of the results is not possible. However, concordant data developed by independent research teams based in Europe and in the US, using different experimental strategies, provide reassurance on the reliability of these results. In fact, these two studies, which used robust statistical algorithms for the mutation analysis, show minor discrepancies and several corroborating findings.

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In both studies the number of non-synonymous gene mutations was smaller than in other cancers. Lo Iacono *et al.* found that 20/52 "cancer" genes studied harbored variations in 25/123 (20%) of FFPE biopsies (including intronic, synonymous, non synonymous and regulative mutations). These mutations were clustered in the two main "p53/DNA repair" (*TP53*, *SMACB1*, and *BAP1*) and "PI3K-AKT" (*PDGFRA*, *KIT*, *KDR*, *HRAS*, *PIK3CA*, *STK11* and *NF2*) pathways.

Guo *et al.* instead sequenced "only" the exome, but looked for all possible genes, and found 490 mutated genes, of which 447 (97%) were mutated only in one biopsy, and found an average of 23 mutations per biopsy (range 2–51).

Several factors may explain the different mutation frequency detected: the different source of DNA (FFPE[1] versus frozen biopsies[2]), different platforms, study design and algorithms used, and the fact that Lo Iacono *et al.*[1] sequenced also the intronic regions (except for *BAP1* and *NF2*). The genes most commonly mutated in both studies were *BAP1*, *NF2*, and *CDKN2A*.

Both studies detected frequent somatic non-synonymous *BAP1* mutations in 41%[2] and 58%[1] of MMs respectively, confirming previous findings that detected somatic *BAP1* mutations in 22–61% of MMs[4–6]. These mutations result in stop codons that produce truncated BAP1 proteins lacking the nuclear localization sequence (NLS) or are mutations within the catalytic subunit that impair BAP1 auto-deubiquitination, which is required for nuclear localization[7]. Therefore, these mutations are predicted to result in BAP1 proteins that cannot migrate to the nucleus and which may have aberrant de-ubiquitinase activity in the cytoplasm[8]. Accordingly, Lo Iacono *et al.* found that 52% of 116 MM biopsies stained for nuclear BAP1 – an indication of normal BAP1 activity – while 48% did not, an indication of mutated BAP1[1]. Nuclear BAP1 staining correlated with presence/absence of DNA mutations (p=0.001). Both studies[1, 2] report frequent mutations of *NF2*, encoding Merlin, a component of the Hippo signaling pathway[9], with about 50% frequency, a value comparable with previous reports[10, 11]. Surprisingly, 92% of the specimens analyzed by Lo Iacono *et al.*, showed NF2 expression by immunohistochemistry[1]. The Authors propose that the genetic variations detected might deregulate NF2 without affecting protein expression and stability. Alternatively, the *NF2* genetic mutations detected are often of minor biological significance.

Lo Iacono *et al.* emphasize the possible role of mutations found in the *PIK3CA* gene encoding the catalytic subunit of PI3K in favoring tumor progression. However, the Authors acknowledge the need to study larger cohorts before conclusions can be drawn.

Guo *et al.* performed an integrative pathway analysis of somatic mutations and focal SCNAs: most recurrent alterations were in the MAPK and Wnt signaling pathways, and in the cell cycle, with recurrent alterations of *CUL1*, *CDKN2A* and *TP53*, the last two matching the results obtained by Lo Iacono *et al.*.

In summary, in spite of the different study design and methodology, these two NGS analyses of the MM genome reveal that inactivating mutations occur randomly and are rarely shared among MM biopsies, with the exception of *BAP1*[1, 2] and to a lesser extent *NF2*, *CDKN2A*[1, 2] and possibly *CUL1*[2].

These results are in agreement with a large body of research that led to the conclusion that driver mutations (i.e., gene mutations present in all tumor cells in most cancers of the same type) are rare; a finding that significantly complicates the attempt to develop target therapies. In fact, the main goal of NGS tumor studies is to identify somatic driver mutations that would become potential therapeutic targets and/or clinical biomarkers. Instead, the emerging picture indicates that each single tumor has its own specific sets of genetic alterations. In addition to inter-tumor genetic heterogeneity, there is also significant intra-tumor genetic heterogeneity as branched evolutionary growth generates genetic diversity in several tumor sub-clones. Thus, a single biopsy taken at a given time point is unlikely to be representative of the full spectrum of tumor genetic alterations. For example, over 60% of mutations detected by NGS in clear cell carcinomas of the kidney were not present in all tumor areas sampled[12]. In the same study, mTOR mutations were found in seven of eight primary sites, but in none of three metastases[12]. The studies of Lo Iacono *et al*. and of Guo *et al*. did not explore the issue of intra-tumor genetic heterogeneity. Based on studies in other tumor types[12], and the recent findings that MMs are heterogeneous from start because they originate as polyclonal malignancies[13], it is easy to predict that MMs will also show marked intra-tumor heterogeneity, further complicating attempts to develop molecular therapies that may benefit a large number of patients.

However, and in spite of these considerations, the studies of Lo Iacono *et al*. and Guo *et al*. have succeeded in identifying recurrent genetic alterations in MMs, that may be "actionable", *BAP1* being the most common. BAP1 is a nuclear ubiquitin carboxy-terminal hydrolase (UCH), associated with multiprotein complexes regulating key cellular pathways, including the cell cycle, cellular differentiation, cell death, gluconeogenesis and DNA repair[8]. When mutated in the germline, carriers develop MMs, uveal and cutaneous melanomas, renal and cholangiorcarcinomas, other malignancies, and often several cancers in combination[5, 8]. Moreover, several studies point at *BAP1* as harboring the putative driver mutations for sporadic (non-genetically related) MMs. We[5] and Bott *et al*.[4], initially reported — in independent and parallel studies using Sanger sequencing from MM biopsies — that 22% and 23% of MM biopsies contained somatic *BAP1* mutations. Yoshikawa *et al*.[6] reported that 61% of cell cultures derived from MM biopsies contained *BAP1* mutations. Arzt *et al*. found that 60% of MM did not stain for nuclear BAP1, suggestive of inactivating mutations[14]. To address the discrepancy in the frequency of *BAP1* mutations detected in different studies, we used an integrated genomic approach to study frozen MM biopsies which included Sanger sequencing, Multiplex Ligation-Dependent Probe Amplification (MLPA), cDNA sequencing, copy number analyses, methylation studies of the *BAP1* promoter and immunohistochemistry. We found that 14/22 (63.6%) MM biopsies contained *BAP1* mutations/inactivation. None of these methodologies alone was able to capture all inactivating BAP1 mutations. Thus, studies using methodology based exclusively on one type of molecular approach, such as Sanger sequencing[4, 5] or NGS[1, 2], will underestimate the percent of MMs carrying *BAP1* mutations. However, BAP1 nuclear staining was detected only in the 8 specimens demonstrated to contain wild-type *BAP1* by the integrated genomic approach described above. Therefore, immunohistochemistry appeared to be capable of capturing the whole array of possible mechanisms of BAP1 inactivation[15].

Several findings underscore the apparent "driver" role of *BAP1* in MMs and point at BAP1 as a potentially useful target: 1) Multiple studies, including these two recent NGS[1, 2] studies, found that *BAP1* is frequently mutated in MM; 2) The persistence of *BAP1* mutations in early pre-malignant lesions as well as in MM biopsies established in cell culture, suggesting that BAP1 mutations are an early event and that MM cells do not select against BAP1 mutations[8]; 3) The finding that, with rare exceptions, BAP1 nuclear staining is either detected in 100% of MM cells, or it is not detected at all[15]. Moreover, frequent somatic mutations in *BAP1* are present in several malignancies[8], making therapies to restore BAP1 activity in tumors relevant to many cancer patients.

Acknowledgments

References

1. Lo Iacono M, Monica V, Righi L, et al. Targeted next-generation sequencing of cancer genes in advanced stage malignant pleural mesothelioma: a retrospective study. J Thorac Oncol. 2014

2. Guo G, Chmielecki J, Goparaju C, et al. Whole exome sequencing reveals frequent genetic alterations in BAP1, NF2, CDKN2A and CUL1 in malignant pleural mesothelioma. Cancer Res. 2014

3. Stratton MR, Campbell PJ, Futreal PA. The cancer genome. Nature. 2009; 458:719–724. [PubMed: 19360079]

4. Bott M, Brevet M, Taylor BS, et al. The nuclear deubiquitinase BAP1 is commonly inactivated by somatic mutations and 3p21. 1 losses in malignant pleural mesothelioma. Nat Genet. 2011; 43:668–672. [PubMed: 21642991]

5. Testa JR, Cheung M, Pei J, et al. Germline BAP1 mutations predispose to malignant mesothelioma. Nat Genet. 2011; 43:1022–1025. [PubMed: 21874000]

6. Yoshikawa Y, Sato A, Tsujimura T, et al. Frequent inactivation of the BAP1 gene in epithelioid-type malignant mesothelioma. Cancer Science. 2012; 103:868–874. [PubMed: 22321046]

7. Mashtalir N, Daou S, Barbour H, et al. Autodeubiquitination protects the tumor suppressor BAP1 from cytoplasmic sequestration mediated by the atypical ubiquitin ligase UBE2O. Mol Cell. 2014; 54:392–406. [PubMed: 24703950]

8. Carbone M, Yang H, Pass HI, et al. BAP1 and cancer. Nat Rev Cancer. 2013; 13:153–159. [PubMed: 23550303]

9. Li W, Cooper J, Zhou L, et al. Merlin/NF2 loss-driven tumorigenesis linked to CRL4(DCAF1)-mediated inhibition of the hippo pathway kinases Lats1 and 2 in the nucleus. Cancer Cell. 2014; 26:48–60. [PubMed: 25026211]

10. Bianchi AB, Mitsunaga SI, Cheng JQ, et al. High frequency of inactivating mutations in the neurofibromatosis type 2 gene (NF2) in primary malignant mesotheliomas. Proc Natl Acad Sci U S A. 1995; 92:10854–10858. [PubMed: 7479897]

11. Sekido Y, Pass HI, Bader S, et al. Neurofibromatosis type 2 (NF2) gene is somatically mutated in mesothelioma but not in lung cancer. Cancer Res. 1995; 55:1227–1231. [PubMed: 7882313]

12. Gerlinger M, Rowan AJ, Horswell S, et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. N Engl J Med. 2012; 366:883–892. [PubMed: 22397650]

13. Comertpay S, Pastorino S, Tanji M, et al. Evaluation of clonal origin of malignant mesothelioma. J Transl Med. 2014; 12:301. [PubMed: 25471750]

14. Arzt L, Quehenberger F, Halbwedl I, et al. BAP1 protein is a progression factor in malignant pleural mesothelioma. Pathol Oncol Res. 2014; 20:145–151. [PubMed: 23963927]

15. Nasu M, Emi M, Pastorino S, et al. High Incidence of Somatic BAP1 Alterations in Sporadic Malignant Mesothelioma. Journal of Thoracic Oncology. 2015 Accepted for publication pending minor revisions.