Molecular & Cellular Oncology 2:2, e965631; January 1, 2015; Published with license by Taylor & Francis Group, LLC

Do changes in the *c-MYC* coding sequence contribute to tumorigenesis?

Abhishek A. Chakraborty[1,2] and William P. Tansey[1,*]

[1]Department of Cell and Developmental Biology; Vanderbilt University School of Medicine; Nashville, TN USA; [2]Present address: Department of Medical Oncology; Dana-Farber Cancer Institute and Brigham and Women's Hospital; Boston, MA USA

Keywords: MYC, proteolysis, cancer, lymphomagenesis, mutation

The role of changes in the *c-MYC* coding sequence in cancer is controversial. Overexpression of wild-type protein is sufficient to drive tumorigenesis, yet point mutations in *c-MYC* are common in Burkitt's lymphoma. Our discovery that disparate tumor-associated mutations in *c-MYC* have similar protumorigenic effects suggests that these mutations contribute directly to malignancy.

c-MYC (hereafter called MYC) is an oncoprotein transcription factor that is broadly deregulated in a slew of malignancies.[1] Unlike many other oncoproteins (e.g., RAS), tumorigenic activation of MYC does not require changes to the coding sequence, and indeed almost all cancers express pristine forms of MYC protein. Instead, MYC becomes oncogenic when overexpressed as a result of events such as gene amplification, rearrangements, or transcriptional induction. The rarity of MYC mutations in cancer, and the overwhelming experimental evidence that simply increasing MYC levels puts cells on the path to tumorigenesis, has led to the conclusion that changes to the MYC protein sequence are not part of the landscape of human cancer.

That said, MYC mutations are common in one specific type of cancer—Burkitt's lymphoma (BL). The presence of mutations in this context is surprising because BL patients carry a chromosomal translocation (8;14) that places *MYC* transcription under the control of the immunoglobulin μ heavy chain enhancer, driving high levels of MYC synthesis in B cells. As early as 1993, it was reported that up to 50% of BL patients carry point mutations that alter the coding sequence of the translocated *MYC* allele.[2] These mutations are spread throughout the primary *MYC* sequence, but tend to cluster at sites within the first 150 amino acids of the protein, a region termed a 'degron' that signals MYC destruction by ubiquitin (Ub)-mediated proteolysis[3] (**Fig. 1**). Accordingly, the handful of tumor-associated MYC mutants that have been tested are more stable than the wild-type protein,[3] and other groups have shown that select mutations in the highly-conserved "Myc box I" (MbI) region of MYC (e.g., threonine 58 to alanine; T58A) subvert MYC proteolysis by disabling its interaction with the SCF^{Fbw7} Ub-ligase complex.[4] Perturbing destruction of MYC by this pathway has real consequences for MYC function, as MYC mutants such as T58A are much more tumorigenic in mice and drive cancer without selecting for loss of p53-dependent tumor surveillance mechanisms,[5] providing tantalizing evidence that these mutations enhance MYC function.

Despite their prevalence in BL and their documented effects on MYC stability and activity, the significance of tumor-associated *MYC* mutations has been difficult to discern. On one hand, *MYC* mutations are not typically seen in other cancers, and as the 8;14 translocation places *MYC* in a hypermutable region of the genome it is tempting to conclude that such events are simply 'collateral damage' with no consequence for tumorigenesis. This notion is further supported by the fact that MYC is vastly overexpressed in BL cells, raising the issue of whether further increasing MYC expression (by disabling its proteolysis) could have any impact on cancer-relevant processes. On the other hand, the recurring nature of these mutations, their clustering to select regions of MYC, and their effects on MYC behavior all suggest that some pressure is at work to select for these mutations in BL. How can this controversy be resolved?

A limitation of studies performed to date in this area (including our studies) is that only a few tumor-associated MYC mutants have been characterized in detail, all of which cluster within MbI. If MYC mutations are relevant to BL, we would expect that other recurring mutations in MYC—outside of MbI and the amino terminus—would behave similarly to mutants such as T58A, stabilizing MYC and rendering it aggressively oncogenic. Although such mutations have been hard to find in the past, recent tumor resequencing efforts[6–8] have greatly expanded the number of mutant *MYC* alleles that have been sequenced, allowing us to

*Correspondence to: William P. Tansey; Email: william.p.tansey@vanderbilt.edu
Submitted: 08/04/2014; Revised: 08/12/2014; Accepted: 08/13/2014
http://dx.doi.org/10.4161/23723548.2014.965631

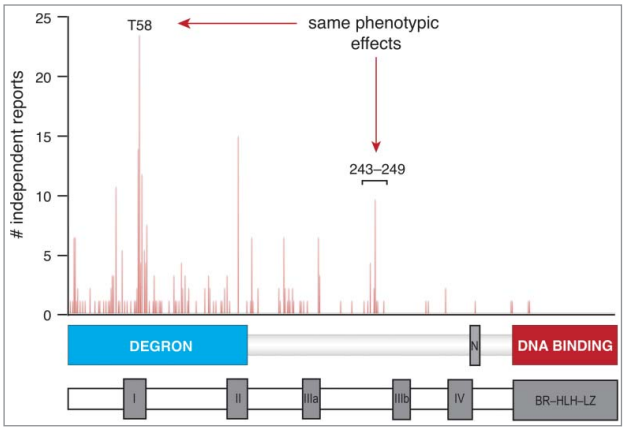

Figure 1. Tumor-associated mutations in *c-MYC*. The figure shows the distribution and frequency of BL tumor-associated mutations in *c-MYC*, indicating the number of independent identifications of mutations at each position. Beneath the graph are 2 cartoons of the c-MYC protein showing (top) the amino-terminal degron, the nuclear localization signal ('N'), and the C-terminal DNA binding domain, and (bottom) conserved 'MYC boxes' I to IV, as well as the conserved basic region-helix-loop-helix-leucine zipper at the carboxyl-terminus of the protein (BR-HLH-LZ). Residue threonine 58 (T58), the most common site of tumor-associated mutations, and the 243–249 cluster that we recently described are identified.

identify a previously unrecognized hotspot for mutations located at residues 243–249, distal from the amino terminus (**Fig. 1**). Our analysis of the most commonly recurring mutation in this region, proline 245 to alanine (P245A), showed that it precisely phenocopies T58A[9] in terms of stabilizing MYC, activating MYC function *in vitro*, and allowing MYC to drive tumorigenesis without the need for collaborating p53 loss.[9] The striking similarity in the effects of tumor-associated mutations in disparate regions of MYC strongly implies that a common molecular process selects these mutants in BL, and in turn suggests a relevance of these mutations to tumor onset or progression.

The issue of whether mutations in MYC contribute to disease is not simply an intellectual exercise. If tumor cells select for mutations that subvert Ub-mediated destruction of MYC, the implication is that interaction of MYC with the Ub-proteasome system plays a pivotal role in regulating MYC function. Amid the backdrop of massive MYC overexpression seen in BL cells, however, it is difficult to imagine how further bolstering MYC levels could promote tumorigenesis, and even more difficult to imagine how such an increase could make MYC inherently less apoptotic. Instead, we hypothesize that these mutations qualitatively alter MYC function, possibly by disrupting MYC interaction with the ubiquitylation/destruction machinery. Moreover, the fundamentally altered behavior of MYC tumor mutants reveals that there are in fact 2 classes of BL patient—those with MYC mutations and those without—whose disease may have been caused by a common translocation but, because of mutations in MYC, have fundamentally different properties and response to therapies. It is possible, for example, that BL expressing mutant MYC has intact apoptotic responses and might benefit from treatments designed to induce apoptosis—treatments that may fail in BL patients expressing wild-type MYC, where selective pressures have resulted in loss of the apoptotic machinery. Further efforts to study tumor-derived MYC mutants, therefore, could very well reveal novel mechanisms controlling MYC function and inform therapeutic strategies to treat a large percentage of BL patients.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Tansey WP. Mammalian MYC proteins and cancer. New Journal of Science 2014; 2013:1-27; http://dx.doi.org/10.1155/2014/757534
2. Bhatia K, Huppi K, Spangler G, Siwarski D, Iyer R, Magrath I. Point mutations in the c-Myc transactivation domain are common in Burkitt's lymphoma and mouse plasmacytomas. Nat Genet 1993; 5:56-61; PMID:8220424; http://dx.doi.org/10.1038/ng0993-56
3. Salghetti SE, Kim SY, Tansey WP. Destruction of Myc by ubiquitin-mediated proteolysis: cancer-associated and transforming mutations stabilize Myc. Embo J 1999; 18:717-26; PMID:9927431; http://dx.doi.org/10.1093/emboj/18.3.717
4. Welcker M, Orian A, Jin J, Grim JA, Harper JW, Eisenman RN, Clurman BE. The Fbw7 tumor suppressor regulates glycogen synthase kinase 3 phosphorylation-dependent c-Myc protein degradation. Proc Natl Acad Sci U S A 2004; 101:9085-90; PMID:15150404; http://dx.doi.org/10.1073/pnas.0402770101
5. Hemann MT, Bric A, Teruya-Feldstein J, Herbst A, Nilsson JA, Cordon-Cardo C, Cleveland JL, Tansey WP, Lowe SW. Evasion of the p53 tumour surveillance network by tumour-derived MYC mutants. Nature 2005; 436:807-11; PMID:16094360; http://dx.doi.org/10.1038/nature03845
6. Schmitz R, Young RM, Ceribelli M, Jhavar S, Xiao W, Zhang M, Wright G, Shaffer AL, Hodson DJ, Buras E, et al. Burkitt lymphoma pathogenesis and therapeutic targets from structural and functional genomics. Nature 2012; 490:116-20; PMID:22885699; http://dx.doi.org/10.1038/nature11378
7. Giulino-Roth L, Wang K, MacDonald TY, Mathew S, Tam Y, Cronin MT, Palmer G, Lucena-Silva N, Pedrosa F, Pedrosa M, et al. Targeted genomic sequencing of pediatric Burkitt lymphoma identifies recurrent alterations in antiapoptotic and chromatin-remodeling genes. Blood 2012; 120:5181-4; PMID:23091298; http://dx.doi.org/10.1182/blood-2012-06-437624
8. Love C, Sun Z, Jima D, Li G, Zhang J, Miles R, Richards KL, Dunphy CH, Choi WW, Srivastava G, et al. The genetic landscape of mutations in Burkitt lymphoma. Nat Genet 2012; 44:1321-5; PMID:23143597; http://dx.doi.org/10.1038/ng.2468
9. Chakraborty AA, Scuoppo C, Dey S, Thomas LR, Lorey SL, Lowe SW, Tansey WP. A common functional consequence of tumor-derived mutations within c-MYC. Oncogene 2014; In press