Editorial

Functional and clinical impact of *MYC* mutations in diffuse large B cell lymphomas

Nathalie A. Johnson

Department of Medicine and Oncology, McGill University, Lady Davis Institute for Medical Research, Jewish General Hospital, Montréal, Canada
Correspondence to: Nathalie A. Johnson. Department of Medicine and Oncology, McGill University, Lady Davis Institute for Medical Research, Jewish General Hospital, 3755, Chemin de la Côte-Ste-Catherine, Montréal, H3T 1E2, Québec, Canada. Email: nathalie.johnson@mcgill.ca.
Comment on: Xu-Monette ZY, Deng Q, Manyam GC, *et al.* Clinical and Biologic Significance of MYC Genetic Mutations in De Novo Diffuse Large B-cell Lymphoma. Clin Cancer Res 2016;22:3593-605.

Submitted Jul 03, 2016. Accepted for publication Jul 15, 2016.
doi: 10.21037/tcr.2016.07.22
View this article at: http://dx.doi.org/10.21037/tcr.2016.07.22

MYC is a transcription factor and an oncogene that drives the pathogenesis of Burkitt lymphomas (BL) and 30–50% of diffuse large B cell lymphomas (DLBCL) (1-3). MYC protein has critical functions that profoundly impact cell fate (reviewed) (4). These include regulation of transcription, translation, metabolism and cell cycle progression (4). Paradoxically, MYC over-expression induces genomic instability and can initiate apoptosis by increasing expression of the tumor suppressor P53 and the pro-apoptotic protein BIM (5). Inactivating P53 or over-expressing the anti-apoptotic protein BCL2 that neutralizes BIM are two effective mechanisms exploited by lymphoma cells to evade MYC-induced apoptosis, leading to unabated cell proliferation and therapeutic resistance (6,7). Mutations in *TP53* and the co-expression of MYC and BCL2 proteins are associated with a poor survival in DLBCL patients treated with chemo-immunotherapy (2,3,8). Therefore understanding the mechanisms that deregulate MYC in aggressive lymphomas is clinically important.

MYC expression in DLBCL is heterogeneous and its mechanism of deregulation differs according to the molecular subtype (3). MYC expression is higher in the activated B cell (ABC) type of DLBCL as a result of transcriptional up-regulation of other oncogenic pathways. MYC expression in the germinal center B (GCB) type is most commonly detected in cases harboring *MYC* translocations that can involve different gene partners. In over half of the cases, the translocation juxtaposes *MYC* upstream of one of the immunoglobulin gene (*IG*) promoters, leading to constitutive transcription of *MYC* mRNA (9). However, the proximity to the *IG* increases the likelihood that *MYC* will become an inadvertent target of somatic hypermutation (SHM) driven by activation induced cytidine deaminase (AID) (10). Over 50% of BLs harbor *MYC* mutations, clustering in "hot spot" regions that appear to be "gain-of function" (5,11). For instance, some mutants increase *MYC* mRNA stability and MYC protein expression (12). They also decrease pro-apoptotic function by preventing the activation of BIM and facilitate lymphoma progression in the absence P53 activation, ultimately leading to more aggressive lymphomas (5). However, the role of *MYC* mutations in DLBCL is unclear. In the February 2016 issue of *Clinical Cancer Research*, Xu-Monette *et al.* have addressed this question (13). They performed Sanger sequencing of the *MYC* gene in 750 well-characterized *de novo* DLBCL samples and report an association between the presence of *MYC* mutations, MYC protein expression and clinical outcome.

Xu-Monette reported that *MYC* mutations were common in DLBCL, being detected in 33% of samples. While this is comparable to a previous report (10), the rate of coding mutations was higher than what has been reported by exome sequencing (16% versus 5%) (10,14). The lack of germline DNA in the Xu-Monette study may in part explain this discrepancy, resulting in over-calling somatic mutations when the *MYC* variants could have been germline single nucleotide polymorphisms (SNPs) (13). Unlike BL, *MYC* mutations in DLBCL were distributed across the entire gene including the 5' and 3' untranslated regions (UTR), which include many regulatory elements. The most common mutations affected amino acid residues T58, S62, S67, P79, R83, F138, A141, P164, S175, and

A185. The T58 or F138 mutations, previously identified as being "gain of function", were only present in 0.5% of the cohort. Thus, the incidence and pattern of *MYC* mutations in DLBCL is different than in BL.

The authors then demonstrated that *MYC* mutations, including SNPs (e.g., N11S), could impact MYC protein expression. Within the 16% of coding mutations, the T58 and F138 variants, known to increase mRNA stability, were associated with very high MYC protein expression. The other coding *MYC* mutations were associated with more variable protein expression. Interestingly, 2% of cases had *MYC* mutations predicting for a dysfunctional or truncated protein and had no or low MYC protein expression by immunohistochemistry (IHC). Within the non-coding mutations, the 5'UTR had the highest mutation rate in the entire gene (~20%). Mutations in this region have been previously shown to increase *MYC* protein expression by preventing the premature block in transcription elongation (15). In the Xu-Monette study, there was a trend for higher MYC protein expression in the 5'UTR mutants [38% versus 31% for MYC wild types (WT)], but this was not statistically significant (P=0.24). There was also no change in MYC protein expression with 3'UTR mutations, present in 6% of cases. Using an *in vitro* model, they then introduced different MYC mutants into the Rat1a cell line to directly assess the effect of mutations on protein expression. These embryonic fibroblast cells have been previously shown to be very sensitive to genomic instability upon transient increases in MYC expression (16). They demonstrated a marked variation in MYC protein expression depending on the mutation, ranging from the highest levels obtained with P57S and the lowest levels obtained with S159R and the SNP N11S. Thus, unlike BL, the *MYC* mutations detected in DLBCL resulted in more variable levels of MYC protein that were lower than the P57/T58 variants or WT MYC.

In addition to affecting MYC protein expression, Xu-Monette showed that *MYC* mutations were associated with other important prognostic factors in DLBCL. There was a significant association between the presence of a *MYC* translocation and *MYC* mutations, features associated with the GCB subtype. However, the presence of a *MYC* translocation is not a requirement for the acquisition of *MYC* mutations because it was only present in a third of the MYC mutant cases. In the ABC cases, *MYC* mutations were associated with the presence of a *BCL6* translocation. Similar to what has been reported in BL, the presence of *MYC* mutations in DLBCL was associated with a WT P53. This is consistent with prior studies that showed that *MYC* mutations disable the pro-apoptotic function of MYC allowing cells to proliferate without having to inactivate *TP53* (5).

With the exceptions of T58 and F138 variants, *MYC* mutations in the Xu-Monette study appeared to be associated with a favorable outcome in DLBCL. One of the biggest strengths of this work is the access to a large clinically-annotated cohort of DLBCL biopsies allowing to detect statistically significant differences in survival based on the presence of mutations that occur in only 0.5% to 2% of patients. T58 and F138 variants, which were associated with high MYC expression, were associated with a significantly inferior outcome. The remaining coding *MYC* mutations correlated with a favorable outcome, though in multivariate analysis, MYC protein expression and not *MYC* mutations, was significantly associated with overall and progression-free survival. Mutations in the 3'UTR were also associated with a poor outcome, though the mechanisms of this are unclear. The authors did not include factors that are known to be associated with a poor survival in their multivariate analysis, such as the co-expression of MYC and BCL2, cell of origin subtype or the presence of *P53* mutations. Taken together, the positive clinical outcome observed in patients with MYC-mutant DLBCL is likely related to its association with known favorable features, such as low MYC protein expression, a GCB phenotype and a WT P53 status.

Given the proliferative advantage of expressing WT MYC, a key question is to understand why 33% of DLBCL harbor mutations in *MYC*. These occur as a consequence of aberrant SHM based on their pattern and features consistent with AID activity (10). Thus they could be beneficial (drivers) or non-deleterious (passengers). In the Xu-Monette study, the MYC mutant (P57S) was a clear driver mutation because it increased protein expression, increased colony formation *in vitro*, decreased apoptosis upon serum withdrawal and increased tumor volumes in immunosuppressed mice. The phenotype of non-P57S mutants appeared to be intermediate between clones expressing WT MYC and the empty vector controls that did not express any MYC. They all had impaired proliferative capacity compared to WT MYC as assessed by cell proliferation and tumor growth potential *in vivo*. However, they were significantly better at resisting apoptosis in the serum deprivation assay compared to WT MYC controls. While the authors conclude that these mutations were "loss of function", these results imply that DLBCLs expressing mutant MYC protein could have a small advantage over DLBCLs that don't express MYC, but not over DLBCLs

Table 1 Effect of *MYC* mutations on protein expression, cell proliferation, apoptosis and outcome

MYC status in DLBCL	Levels of MYC protein expression	Tumor growth	Apoptosis	Response to chemotherapy
P57 or T58 MYC expression[#]	+++	+++	Impaired by MYC MUT (low BIM*); P53 WT	Poor
MYC WT expression	++	++	Selection for TP53 MUT	Poor
Other MYC MUT expression	+/–	+	Impaired by MYC MUT; P53 WT*	Good
No MYC expression	–	–	No effect	Good

The + and – denote the levels of change, with – denoting absence of protein expression and delayed tumor growth and +++ denoting the highest levels of expression and the most rapid tumor growth. DLBCL, diffuse large B cell lymphomas; MUT, mutant; WT, wild type. [#], may include other MYC mutants that also have a proliferative advantage such as F138, but not tested in their *in vitro* assay. *, previously shown by Hemann *et al*. to inhibit apoptosis by preventing activation of BIM. This mechanism may be relevant to the other MYC mutations observed in DLBCL but were not addressed by Xu-Monette *et al*.

that express WT MYC (*Table 1*). More importantly, the major selection advantage of expressing mutant MYC versus WT MYC is to evade apoptosis, a feature consistent with some of these being potential "driver" mutations.

Overall, the work by Xu-Monette *et al.* improves our understanding of the mechanisms that regulate MYC protein expression in DLBCL. Placed in clinical context, it provides a logical explanation as to why some cases harboring a *MYC* translocation don't express high levels of MYC protein. It also implies that lack of MYC protein expression predicts for a favorable outcome, even in the context of a *MYC* translocation that has previously been associated with a poor outcome (17). Taken together, the data support the notion that most *MYC* mutations in DLBCL don't have the prominent "driver" proliferative phenotype that is observed in BL. Rather, they have an intermediate phenotype that lacks the growth advantage conferred by WT MYC, but may nonetheless give DLBCL cells a survival advantage by escaping P53-mediated apoptosis. Given that these MYC mutant DLBCL cells retain WT P53 expression, they can still be sensitive to chemotherapy.

Acknowledgments

Funding: NA Johnson is a recipient of a Canadian Cancer Society Research Institute and Canadian Institute of Health Research grants (703425, 299607). She has received salary support from the Fonds de Recherche en Santé du Québec.

Footnote

Provenance and Peer Review: This article was commissioned and reviewed by the Section Editor Duo Liu, MD (Harbin Medical University Cancer Hospital, Harbin Medical University, Harbin, China).

Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr.2016.07.22). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are 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.

References

1. Dalla-Favera R, Bregni M, Erikson J, et al. Human c-myc onc gene is located on the region of chromosome 8 that is translocated in Burkitt lymphoma cells. Proc Natl Acad Sci U S A 1982;79:7824-7.
2. Green TM, Young KH, Visco C, et al. Immunohistochemical double-hit score is a strong predictor of outcome in patients with diffuse large B-cell lymphoma treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone. J Clin Oncol

2012;30:3460-7.
3. Johnson NA, Slack GW, Savage KJ, et al. Concurrent expression of MYC and BCL2 in diffuse large B-cell lymphoma treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone. J Clin Oncol 2012;30:3452-9.
4. Meyer N, Penn LZ. Reflecting on 25 years with MYC. Nat Rev Cancer 2008;8:976-90.
5. Hemann MT, Bric A, Teruya-Feldstein J, et al. Evasion of the p53 tumour surveillance network by tumour-derived MYC mutants. Nature 2005;436:807-11.
6. Schmitt CA, Lowe SW. Bcl-2 mediates chemoresistance in matched pairs of primary E(mu)-myc lymphomas in vivo. Blood Cells Mol Dis 2001;27:206-16.
7. Lowe SW, Ruley HE, Jacks T, et al. p53-dependent apoptosis modulates the cytotoxicity of anticancer agents. Cell 1993;74:957-67.
8. Xu-Monette ZY, Wu L, Visco C, et al. Mutational profile and prognostic significance of TP53 in diffuse large B-cell lymphoma patients treated with R-CHOP: report from an International DLBCL Rituximab-CHOP Consortium Program Study. Blood 2012;120:3986-96.
9. ar-Rushdi A, Nishikura K, Erikson J, et al. Differential expression of the translocated and the untranslocated c-myc oncogene in Burkitt lymphoma. Science 1983;222:390-3.
10. Pasqualucci L, Neumeister P, Goossens T, et al. Hypermutation of multiple proto-oncogenes in B-cell diffuse large-cell lymphomas. Nature 2001;412:341-6.
11. Bhatia K, Huppi K, Spangler G, et al. Point mutations in the c-Myc transactivation domain are common in Burkitt's lymphoma and mouse plasmacytomas. Nat Genet 1993;5:56-61.
12. 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.
13. Xu-Monette ZY, Deng Q, Manyam GC, et al. Clinical and Biologic Significance of MYC Genetic Mutations in De Novo Diffuse Large B-cell Lymphoma. Clin Cancer Res 2016;22:3593-605.
14. Pasqualucci L, Trifonov V, Fabbri G, et al. Analysis of the coding genome of diffuse large B-cell lymphoma. Nat Genet 2011;43:830-7.
15. Cesarman E, Dalla-Favera R, Bentley D, et al. Mutations in the first exon are associated with altered transcription of c-myc in Burkitt lymphoma. Science 1987;238:1272-5.
16. Felsher DW, Bishop JM. Transient excess of MYC activity can elicit genomic instability and tumorigenesis. Proc Natl Acad Sci U S A 1999;96:3940-4.
17. Savage KJ, Johnson NA, Ben-Neriah S, et al. MYC gene rearrangements are associated with a poor prognosis in diffuse large B-cell lymphoma patients treated with R-CHOP chemotherapy. Blood 2009;114:3533-7.

Cite this article as: Johnson NA. Functional and clinical impact of *MYC* mutations in diffuse large B cell lymphomas. Transl Cancer Res 2016;5(S2):S257-S260. doi: 10.21037/tcr.2016.07.22

