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Published in:
Experimental Hematology

DOI:
10.1016/j.exphem.2018.02.006

Publication date:
2018

Document version:
Accepted manuscript

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Citation for published version (APA):

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PII: S0301-472X(18)30078-X
DOI: https://doi.org/10.1016/j.exphem.2018.02.006
Reference: EXPHEM 3615

To appear in: Experimental Hematology

Received date: 15-1-2018
Revised date: 15-2-2018
Accepted date: 16-2-2018

Please cite this article as: Blerta Green, Alberto Martin, Antoaneta Belcheva, Deficiency in the DNA glycosylases UNG1 and OGG1 does not potentiate c-myc-induced B-cell lymphomagenesis, Experimental Hematology (2018), https://doi.org/10.1016/j.exphem.2018.02.006.

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Deficiency in the DNA glycosylases UNG1 and OGG1 does not potentiate c-Myc-induced B-cell lymphomagenesis

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Abstract

C-Myc overexpression mediates lymphomagenesis, however, secondary genetic lesions are required for its full oncogenic potential. The origin and the mechanism of formation of these mutations are unclear. Using the lacI - mutation detection system, we show that secondary mutations occur early in B-cell development and are repaired by Msh2. The mutations at the lacI gene were predominantly at C:G base pairs and CpG motifs suggesting that they were formed due to cytosine deamination or oxidative damage of G. Hence, we investigated the role of Ogg1 and UNG glycosylases in c-Myc driven lymphomagenesis but found that their deficiencies did not influence the disease outcome in the Eµ c-Myc mouse model. We also show that Rag proteins do not contribute to secondary lesions in this model. Our work suggests that mutations at C:G basepairs that are repaired primarily by the mismatch repair system arise early in B-cell ontogeny to promote c-Myc driven lymphomagenesis.

Introduction

Lymphomas are a group of cancers that occur in lymphoid tissue during lymphocyte development. The majority of the lymphomas (95\%) are from B-cell origin [1] and despite the extensive research focusing on understanding the molecular events leading to this disease, the full mechanistic details still remain elusive. It is now accepted that the natural processes guiding normal B cell activation and differentiation are at the root of B-cell transformation. B cells are highly susceptible to chromosomal translocations mainly due to the fact that the process of antibody diversification involves naturally
induced DNA breaks and mutations [2]. Indeed, the most aggressive B-cell lymphomas are caused by chromosomal translocations that juxtapose the \(c-Myc\) gene near the regulatory elements of the immunoglobulin \(\mu\) heavy chain or \(\lambda\) and \(\kappa\) light chain genes [3, 4]. \(C-Myc\) is a master transcription factor that regulates the expression of genes involved in cellular processes such as proliferation, cell growth, DNA replication, protein biosynthesis, regulation of cell metabolism and apoptosis [5, 6, 7, 8]. The enormous oncogenic potential of \(c-Myc\) has stimulated the search of identification of novel Myc-dependent translocations, its transcription targets and behaviour during carcinogenesis, and possible means for inhibition of its function [9]. In this regard, the \(E\mu\) \(c-Myc\) transgenic mice that overexpress \(c-Myc\) in the B-cell lineage have been used as a model system to investigate the mechanisms driving lymphoma development [10, 11]. In these mice the transgene construct consists of the \(Myc\) oncogene (\(c-myc\)) coupled to the immunoglobulin heavy chain enhancer \(IgH\), named \(E\mu\). As a result the \(c-Myc\) becomes activated specifically in B-lineage, providing enhanced proliferative potential of the cells [12]. Because the \(E\mu\) enhancer is activated early in B-cell ontogeny \(E\mu\) \(c-Myc\) transgenic mice exhibit enlarged population of actively proliferating B-cell precursors in the bone marrow, spleen and lymph nodes. B-cell lymphomas arise in a stochastic manner between 6 and 83 weeks of age. The tumors are derived from different stages of B-cell development and are monoclonal in origin [10, 11]. However, \(c-Myc\) overexpression alone is not sufficient to induce tumor development, indicating that other mutations are required for this process [13, 14]. Indeed, \(c-Myc\)-induced lymphomagenesis is accelerated when the \(c-Myc\) mutation is combined with additional mutations in genes such as \(pim-1, bmi-1, bla-1\) [15], as well as genes that function in ARF-Mdm2-p53 pathway [16, 17]. However, the origin and the mechanism through which these secondary mutations occur are unknown. It has been proposed that both the processes involved in B-cell development and the environment in which B cells mature are inherently mutagenic. Some \(c-Myc\) translocations are mediated by the recombination-activating gene \(RAG1\) [18]. However, we have previously shown that lymphomagenesis was not abrogated in \(RAG1^{-/-}\) \(E\mu\) \(c-Myc\) mice, but found that B-cell progenitors are particularly more susceptible to \(c-Myc\)-induced transformation [19]. We have suggested that the abnormally high incidence of lymphomas in \(RAG1^{-/-}\) \(E\mu\) \(c-Myc\) mice is due to increased level of mutagenic processes that occur during early B-cell development. Therefore, the unusually high increase of mutagenic events during early B-cell development could be a source of the secondary mutations that cooperate with c-
Myc. Such a highly mutagenic environment is defined, for example, by production of reactive oxygen species (ROS) and nitric oxide (NO). Primary B cells rapidly generate ROS that regulate their activation and proliferation [20, 21]. ROS are also produced during normal mitochondrial respiration [22]. However, the prolonged production of ROS can be a source of oxidative DNA damage. Furthermore, in B cells, production of NO by nitric oxide synthase (NOSi and NOS2) enzymes has been shown to play a role in the regulation of the process of class switching to IgA [23]. Therefore, the B-cell environment is extremely mutagenic and the proper function of the DNA repair mechanisms plays a crucial role in repair of the oxidative DNA damage. Hence, the frequency of secondary mutations that cooperate with c-Myc to induce transformation of B cells will be increased by deficiencies of DNA repair. We have shown that Msh2, a central protein in the DNA mismatch repair (MMR) pathway, actively suppresses c-Myc driven lymphomagenesis [17]. The enhanced development of lymphomas observed in Msh2−/− Eµ c-Myc mice is likely due to the enhanced frequency of mutations during haematopoiesis and/or B-cell ontogeny. These mutations could be spontaneous, caused by replication errors, or induced by a mutagen(s) such as ROS and NO, and remain unrepaird due to the Msh2-deficiency [24]. One of the most common types of mutation in B cells that can arise is spontaneous or NO-induced deamination of deoxycytosine that results in uracil [25]. Importantly, MMR pathway engages such G:U mismatches [26] and have been shown to repair NO and ROS induced DNA lesions [27, 28]. Therefore, the enhanced development of lymphomas observed in Msh2−/− Eµ c-Myc mice could be explained by the inability to repair NO and ROS induced DNA lesions. However, Uracil-DNA glycosylase (UNG1) and 8-Oxoguanine DNA glycosylase 1 (Ogg1) are also involved in the repair of NO and ROS-induced DNA damage. Deficiency in UNG1 leads to increased background mutation frequencies at C:G basepairs [29, 30], while oxidized DNA bases, such as 7,8-dihydro-8-oxy-2′-deoxyguanosine (8-oxo-G) are processed by (Ogg1) [31]. These observations let us to hypothesize that UNG1 and Ogg1, similarly to Msh2, may be involved in the suppression of the mutations that complement c-Myc-mediated B-cell transformation. Therefore, deficiencies in these DNA glycosylases would result in accelerated lymphomagenesis in Eµ c-Myc transgenic mice, as we observed under Msh2-deficient background.
In this study, we examined the origin of the secondary mutations in B cells that cooperate with c-Myc during lymphomagenesis. In addition, we investigated the potential role of Ogg1 and UNG1 glycosylases in repair of these mutations.

Materials and methods

Mice

UNG\textsuperscript{+/−} and Ogg1\textsuperscript{+/−} mice, kindly provided by Dr. H. E. Krokan and Dr. J. Stavnezer, respectively, were interbred with the E\textsubscript{μ} c-Myc mouse [10, 11], purchased from Jackson Laboratories (stock number 002728). Msh2\textsuperscript{+/−} mice [32] were provided from Tak W Mak (Ontario Cancer Institute, Toronto). RAG1\textsuperscript{−/−} mice were purchased from Jackson Laboratories (stock number 002216). All mice were on the C57BL/6 genetic background. Tail DNA was used to genotype RAG1\textsuperscript{+/−}, E\textsubscript{μ} c-Myc mice as suggested by Jackson Laboratories. Msh2\textsuperscript{+/−} genotyping was done as described previously [33]. Mice were monitored daily for signs of morbidity and lymphoma. Terminally ill mice were sacrificed, and tumors from various sites were harvested to obtain single cell suspensions and subjected to flow cytometric analysis. The day at which a mouse was either sacrificed or found dead because of lymphoma was used for Kaplan–Meier survival analysis. For mutation analysis we used Big Blue C57BL/6 homozygous male mice that were purchased from Stratagene. RAG1\textsuperscript{−/−}, E\textsubscript{μ} c-Myc, Msh2\textsuperscript{+/−}, E\textsubscript{μ} c-Myc RAG1\textsuperscript{−/−} mice were crossed with Big Blue (BB\textsuperscript{+}) to generate BB\textsuperscript{+}RAG1\textsuperscript{−/−}, BB\textsuperscript{+}E\textsubscript{μ} c-Myc, BB\textsuperscript{+}Msh2\textsuperscript{+/−} and BB\textsuperscript{+} E\textsubscript{μ} c-Myc RAG1\textsuperscript{−/−} offspring. The mice that carry the shuttle vector (BB+) were identified by PCR as described before [34]. All animals were raised under specific pathogen-free conditions. All animal study protocols were approved by the University of Toronto’s Division of Comparative Medicine Committee.

Flow Cytometry analysis

Live lymphocytes were isolated from the single cell suspension of lymphomas and characterized by flow cytometry as we have previously described [17, 19]. We defined mature B-cell lymphomas as B220\textsuperscript{+}, IgM\textsuperscript{+} or surface light (κ or λ) chain\textsuperscript{+} because class switched lymphomas are light-chain positive. Pre B lymphomas were defined as B220\textsuperscript{+} surface IgM\textsuperscript{−} and surface light-chain negative. Most of the tumors were homogeneous. Some tumors however showed mixed phenotype and showed pre B and mature B cell populations. These lymphomas were characterized as mixed lymphomas.
To determine the cell numbers in spleen and bone marrow compartment Rag$^{+/−}$, Rag$^{−/−}$, Eµ c-Myc Rag$^{+/−}$ and Eµ c-Myc Rag$^{−/−}$ mice were sacrificed when 24-27 days old. At this age, the mice did not have any lymphoma development. Cells were isolated from both femur and spleen. Total number of cells was measured by flow cytometry. Cocktail of B220-APC, CD43-biotin, BP1-PE and SA-FITC was used to stain BM cells whereas cocktail composed by B220-APC, CD43-biotin, anti-mouse IgM and SA-PE was used to stain the cell in the spleen. The antibodies were purchased from Southern Biotech or eBioscience. Typically, 1 million cells were analysed with flow cytometry to count the number of pro B cells in each compartment.

**Mutation analysis**

Tissue preparation and transgenic λ phage rescue was carried out as described previously [35]. Briefly, genomic DNA was isolated from bone marrow of BB$^{+}$, BB$^{+}$Msh2$^{−/−}$, BB$^{+}$RAG1$^{−/−}$, BB$^{+}$ Eµ c-Myc RAG1$^{+/−}$, and BB$^{+}$ Eµ c-Myc RAG1$^{−/−}$ mice. Phage was packaged using the Stratagene Transpack Packaging Extract (Sigma) where 10 µL of DNA from each sample was exposed to *in vitro* packaging extract to produce phage in a total final volume of 1mL. First, a dilution assay was performed by mixing 1 µL of the produced phage with 200 µL of SCS-8 bacteria grown to OD$_{600}$=0.5. This mixture was incubated at 37°C water bath for 15 min, mixed with 3-4 mL of molten NZY top agarose (cooled at 50°C) and plated on 10 cm plates. The plates were incubated at 37°C for overnight. On the next day, the number of plaques formed by SCS-8 bacteria were counted and used to determine the total number of phage particles found in the 1mL phage sample. 700 phage particles from each sample were then plated per 15 cm plates in presence of X-gal (phage, 450 µL SCS-8 bacteria of OD$_{600}$=0.5, 8.5 mL molten NZY top agarose, and X gal at final concentration 1.5 mL X-gal/mL sample). The plates were incubated overnight at 37°C and the number of blue plaques (mutants) over the total number of plaques were counted to determine the mutation frequency. Sectored plaques, arising from bacterial processing of DNA damage acquired within the murine host, were not included in the results (i.e. only plaques that were at least 50% blue were counted). For analysis of the specific mutations at the lacI gene the blue plaques were isolated and DNA was sequenced.

**Statistical analysis**

Data were analysed with GraphPad Prism Version 6.0, in which we performed 2-tailed
Student t tests.

Results

**MMR pathway repairs mutations occurring in early stages of B-cell development**

Earlier work showed that constitutive expression of c-Myc is not sufficient to induce tumors and that secondary genetic lesions are required [10, 13, 14]. We previously showed that arresting B cells at the pro/pre B cell stages using either Rag1−/− or the μMT−/− deficient mice leads to accelerated lymphomagenesis in the Eμ c-Myc-transgenic background [17]. This effect could be due to at least two possibilities. First, arresting B cells at a precursor stage might increase the background mutation frequency. Second, mutations that lead to the arrest and accumulation of B-cell progenitors increase the pool of cells subject to transformation events. Because Eμ c-Myc Msh2−/− mice developed more rapidly B-cell lymphoma than their Msh2-sufficient controls, we investigated the hypothesis that this is due to an increased mutation frequency in pro B cells. We found that the mutant frequency in pro B cells (WT) was at ~0.5 x 10−4 (Figure 1), which is similar to the mutant frequency previously measured in follicular B cells and macrophages [34]. However, Msh2−/− pro B cells had a ~4-fold increase in the mutant frequency compared to WT, Rag−/−, Eμ c-Myc Rag−/− and Eμ c-Myc Rag−/− pro B cells (Figure 1), supporting the hypothesis that the MMR pathway repairs mutations in B cells. In addition, we noted that the mutant frequency in Msh2−/− pro B cells (i.e. ~4 x 10−4) was also similar to the previously reported mutant frequency in Msh2−/− follicular B cells and Msh2−/− macrophages [34]. Therefore, the similarities regarding the mutant frequency in B cells and in the myeloid lineages suggests that most of these mutations had occurred and accumulated at early stages, prior to B-cell commitment into the lymphoid or myeloid lineages.
To investigate the nature of the mutations that occur early in B-cell ontogeny, we assessed the spectrum of mutations found at the *lacI* gene in pro B cells since spontaneous mutations, mutagens, or errors-causing processes give characteristic mutations. Hence, we sequenced the blue plaques and analysed the specific type of mutations (Table 1). We found that the majority of the mutations in Msh2+/− pro B cells were at G:C bases pairs and at CpG motifs (Table 1), indicating that these mutations likely originate from cytosine deamination. These type of DNA lesions may arise from spontaneous cytosine deamination events or can be also induced by NO or ROS [25, 27]. Taken together these results indicate that the secondary mutations that work together with c-Myc to induce lymphomagenesis arise in pro B cells and are repaired by the MMR pathway.

**The secondary mutations that cooperate with c-Myc to transform precursor B cells are independent from RAG**

Previously we reported that Eµ c-Myc Rag1−/− mice are more susceptible to lymphoma than Eµ c-Myc Rag+/- mice [19]. This observation let us to suggest that genetic mutations that lead to the arrest and accumulation of B-cell progenitors increase the pool of cells subject to transformation events. To test this notion, we measured the total pro B-cell numbers in both spleen and bone marrow of Eµ c-Myc Rag−/− mice and compared them with the total number of pro B cells in their wild type littermates. We measured significantly more pro B cells in the Eµ c-Myc Rag−/− mice compared to Eµ c-Myc Rag+/- mice in both bone marrow and spleen (Figure 2). Furthermore, we found no difference in the mutation frequency in pro B cells of both Eµ c-Myc Rag−/− and Eµ c-Myc Rag+/- mice (Figure 1). Collectively, these results suggest that RAG proteins do not provide the secondary mutations that collaborate with c-Myc to transform precursor B cells.
Loss of Ogg1 and UNG1 is not critical for c-Myc-induced lymphomagenesis.

The fact that most of the mutations occur in the absence of a functional MMR pathway, i.e., Msh2\(^{-/-}\) pro B cells (Figure 1), implicates that highly mutagenic processes are involved in lymphoma development. We proposed that the rapid proliferation of pro B cells in bone marrow and elevated production of ROS and NO in this tissue could be the source of the secondary mutations that cooperate with c-Myc. Although Msh2 is capable of repairing mutations caused by ROS, along with many other mutations [28], we suggested that some other DNA repair enzymes also play a role in processing such mutations, thereby implicating these as tumor suppressors. One such candidate is Ogg1, which repairs ROS generated 8-oxoguanine [31]. To investigate the possibility that Ogg1 repairs oncogenic mutations in pro B cells, we created E\(\mu\) c-Myc Ogg1\(^{+/}\) mice and compare their susceptibility to lymphoma with E\(\mu\) c-Myc Ogg1\(^{+/-}\) controls. Although the Kaplan-Meyer survival curves revealed that Ogg1-deficiency does not affect the disease susceptibility in these mice (Figure 3a), we further investigated whether the cellular composition of the tumors that were formed in Ogg1-deficient mice were different from the tumors of their WT littermates.
We found that the tumor types seen in 38 Eµ c-Myc Ogg1−/− and 20 Eµ c-Myc Ogg1+/− mice were no different (Figure 3b), suggesting that ROS and NO-induced mutations during early B-cell development are not critical for their transformation, or another enzyme(s) is responsible to repair oxidative DNA damage.

As shown in Table 1, the majority of the mutations in pro B cells occur at G:C bases pairs and at CpG motifs, indicating that they arise from cytosine deamination. Because base excision repair (BER) pathway specialises in the repair of the cytosine deamination, we hypothesized that this pathway plays an important role in the repair of the mutations arising in pro B cells. Hence, we created Eµ c-Myc UNG−/− mice. However, we found that the overall survival of Eµ c-Myc UNG+/− is similar to Eµ c-Myc UNG−/− mice, indicating that UNG-deficiency, similarly to Ogg1 deficiency, did not increase c-Myc-induced lymphomagenesis (Figure 4).

Taken together, our results suggest that secondary mutations that are critical for enhanced c-Myc lymphomagenesis arise in pro B cells. These mutations originate from spontaneous cytosine deamination and/or could be induced by specific deaminases. While a functional MMR pathway is critical for repair of these mutations, our results indicate that DNA glycosylates OGG1 and UNG1 have no role in c-Myc-induced B-cell lymphomagenesis.
Discussion

Overexpression of c-Myc in the B-cell compartment leads to aggressive lymphoma development, however, the requirement of secondary mutations for disease manifestation has been suggested [8, 10, 14]. Genes such as Bmi1 [36], Cbx7 [37], ID3, TCF3, CCND3, BCL2, EZH2, CREBBP, EP300 [38] are found mutated in lymphoma. It has been proposed that these mutations are required to fully elicit the c-Myc transforming potential during lymphomagenesis. A typical example is EZH2, a histone methyltransferase that regulates the methylation status of H3K27 and has been found frequently mutated in B-cell lymphomas [39]. Later it was shown that EZH2 alone does not contribute to lymphoma development but rather cooperates with c-Myc to induce the disease [40]. These findings therefore, point out that secondary mutations occur during B-cell development and are critical for the accelerated c-Myc-driven lymphomagenesis. However, the exact enzyme or factor that mediates the molecular process of formation of these secondary mutations still remains unknown.

Here we asked where do these other secondary mutations come from and what enzyme or process is responsible for secondary oncogenic lesions in B-cell lymphomas. We have previously shown that genetic deficiencies causing arrest in B-cell development and accumulation of B-cell progenitors lead to accelerated lymphomagenesis in Eµ c-Myc transgenic mice [17, 19]. MMR function is critical in the repair of mutations induced during somatic hypermutation (SHM) and class switch recombination (CSR) [41]. Specifically, MMR–deficiency has been associated with accumulation of somatic mutations in various tissues including B cells, and therefore, it was suggested as a candidate mechanism that plays a key role in the accelerated lymphomagenesis in Eµ c-Myc transgenic mice [42, 43]. Here we show the importance of Msh2 protein in the repair of the secondary mutations arising in B cells. The mutation frequency in Msh2-deficient pro B cells was significantly increased compared to WT (Figure 1). We noticed that the mutation frequency in Msh2-deficient pro B cells was similar to the mutation frequency measured in follicular B cells and germinal centre B cells [34], indicating that the majority of the mutations arise early in B-cell development. These results are in agreement with previous data showing that Msh2-dependent MMR function actively suppresses c-Myc-associated oncogenesis [17].

RAG proteins are known to induce DNA breaks that facilitate chromosomal translocations and thereby stimulate lymphomagenesis [18, 44]. These observations led
us to hypothesize that cellular processes typical for precursor B-cell development are mutagenic and/or the pro B-cells environment is enormously mutagenic. To test this notion, we first examined the role of RAG1 in providing secondary mutations. Our results, however, indicate that the mutation frequency in pro B cells was not affected in RAG\(^{-/-}\) mice (Figure 1). The role of RAG proteins in accelerating c-Myc lymphomagenesis has been controversial. Smith and colleagues demonstrated that RAG1-deficient vavP-MYC17 mice were less prone to lymphoma development compared to their RAG1-sufficient controls [45]. Opposite to these findings, we have previously observed that RAG1-sufficient \(\text{E}_\mu\) c-Myc Tg mice have longer lifespan than RAG1-deficient \(\text{E}_\mu\) c-Myc Tg littermates [19]. One possibility for the increased lymphomagenesis in RAG1-deficient \(\text{E}_\mu\) c-Myc Tg mice is that the majority of B cells are blocked at a stage of development where these cells are highly exposed to transformation events. Interestingly, however, we found more pro B cells in the \(\text{E}_\mu\)-c-Myc \(\text{Rag}^{+/}\) mice compared to \(\text{E}_\mu\)-c-Myc \(\text{Rag}^{-/-}\) mice (Figure 2). This result combined with the analysis of the mutation frequency (Figure 1) in the same mice suggests that RAG proteins do not contribute to accumulations of secondary mutations that manifest c-Myc lymphomagenesis in this model.

In this study we also found that the majority of the mutations observed at the \(\text{lacI}\) gene in pro B cells were at G:C bases pairs, and at CpG motifs (Table 1), indicating that mutations in pro B cells probably arise due to cytosine deamination. It is known that deamination of cytosines could be spontaneous [30], induced by ROS [31], or caused by a cytosine deaminase (e.g. Apobec3B). The hematopoietic stem cells are exposed to very low levels of ROS in the bone marrow. Subsequent exposure to elevated concentrations of ROS is an important signalling mechanism for their proliferation and differentiation [46, 47]. ROS can be also induced by the overexpression of the c-Myc oncogene [48]. This directly implicates a role of MMR pathway that is known to process deaminated cytosines [49, 50]. We showed elevated mutation frequency in \(\text{Msh2}^{-/-}\) pro B cells (Figure 1). However, other DNA repair pathways could also contribute to the repair of the ROS-induced mutations. Spontaneous deamination of cytosine generates pro-mutagenic U:G mispairs which have been previously shown to be processed by Ogg1 and UNG [30, 31, 51]. Together with Msh2, UNG is also critical for repair of the mutations induced during SHM and CSR [52]. Mutations in the genes that are involved in the repair of oxidative stress have been linked to cancer
development. Specifically, polymorphisms in DNA base excision gene OGG1 have been associated with the risk of childhood acute lymphoblastic leukemia [53] and lymphoma in humans [54] and mouse models [55]. In addition, UNG has not only been implicated in lymphoma development [29] but also appears a critical enzyme that can be targeted in treatment of lymphoma [56]. While there is no doubt that both Ogg1 and UNG play a role in B-cell lymphomas, the mechanisms through which these enzymes exert protection are not fully understood. In this regard our study has investigated the possibility that these enzymes might be also critical in the repair of secondary mutations that cooperate with c-myc. However, our results showed that the loss of neither of Ogg1 or UNG enzymes affected the survival of the mice compared to their WT littermates (Figure 3a and Figure 4). This indicates either that the deficiency of these enzymes have no role in the generation of the secondary mutations necessary for c-Myc-driven lymphomagenesis or their loss is covered by other DNA repair mechanism such as MMR. It will be interesting to also examine the involvement of MBD4 glycosylase [57] which could be involved in the processing of the G:T and G:U DNA damage [58] and hence, may play a role in c-Myc mediated lymphomagenesis. The removal of uracil can be achieved not only by UNG but also by the action of SMUG1, another glycosylase enzyme operating during BER. The evidence suggests that SMUG1 can compensate the loss of UNG [59]. Furthermore, it was demonstrated that UNG mediated repair of uracil is Msh2 dependent, indicating cooperation between MMR and BER during CSR [60]. It will be not surprising that the loss of UNG in Eµ c-Myc UNG⁻/⁻ mice was compensated by MMR.

Acknowledgements

We thank Maribel Berru for technical help, and to the Martin laboratory for helpful discussions. This research is supported by studentships from the Canadian Institutes of Health Research to B.G. and a grant from the Canadian Cancer Society (703185) to A.M.

References


Figure 1. Frequency of mutations at lacI gene in Pro B cells isolated from bone marrow of mice. Pro B cells were sorted from bone marrow of mice with indicated genotypes (3 mice per genotype), and the mutation frequency at the lacI gene was measured by Big blue mutation screen. Statistical analysis involved a 2-tailed t test, where *p= 0.0158.
Figure 2. Pro B total qcell numbers in spleen and bone marrow compartment.

Total number of cells was measured in both spleen (A) and bone marrow (BM) (B) of mice with the following genotypes: RAG+/−, n=5; RAG−/−, n=5; Eμ c-Myc RAG+/−, n=3; Eμ c-Myc RAG−/−, n=2. One million cells from each mouse were analyzed with flow cytometry to count the number of pro B cells in each compartment. Statistical analysis involved a 2-tailed $t$ test, where *$p<0.05$; ***$p<0.0001$. 
Figure 3. Ogg1 deficiency has no effect on c-myc induced lymphomagenesis. (A) Kaplan-Meier curves show the overall survival of Eµ c-myc Ogg1+/− and Eµ c-myc Ogg1−/− mice. n indicates the number of experimental mice in each group. (B) Tumor characteristics in terminally ill Eµ c-myc from indicated genotypes. The frequency of Pro/Pre B cells, immature/mature B cells or mixed tumors was determined by flow cytometry analysis. Typically, we defined IgD+ IgM+ κλ+ B220+ CD3− cells as mature/immature B cells, whereas IgD− IgM− κλ− B220+ CD3+ cells were designated as pro B (BP-1−) or pre B (BP-1+) cells. The number of lymphomas involved in the assay is indicated on the top of the bars.
Figure 4. UNG does not affect the c-myc induced lymphomagenesis. Kaplan-Meier curves show the overall survival of Eμ c-myc UNG^{+/−} and Eμ c-myc UNG^{−/−} mice. n indicates the number of experimental mice in each group.
Table 1. Mutations found within lacI gene in wild type and Msh2−/− pro B cells

<table>
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<tr>
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<th>BB*Msh2−/− pro B cell (n=3)</th>
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<tr>
<td>Deletions</td>
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<td>5 (23)</td>
</tr>
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</table>

* Refers to the number of animals included into the assay.

1 Percentage of mutations were calculated from total mutations (i.e. base substitutions and insertion/deletion mutations).

2 Mutations at CpG include transition and Transversion mutations at dC on either DNA strand.
Fig 2.tif