Regulation of DNA Repair and Recombination by Mre11 and Cis- Acting Elements in B Lymphocytes

by

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Preface

According to the American Cancer Society, Non- Hodgkin Lymphoma (NHL) is one of the most common cancers in the United States, making up about 4% of all cancers. 85% of these originate in B lymphocytes. A number of these tumors are caused by genome rearrangements that happen as a result of incorrect repair to mistakes made in the DNA known as genome instability. My thesis attempts to understand roles for proteins involved in stabilizing the genome, and to elucidate their importance in the prevention of diseases such as Non- Hodgkin Lymphoma. It is my hope that this research can one day contribute to the discovery of cancer therapeutics aimed at targeting the DNA damage response in malignant cells.

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List of Abbreviations

53BP1 – p53 binding partner 1

AID – activation induced cytosine deaminase

A-T – ataxia telangiectasia

ATLD – ataxia telangiectasia like disorder

ATM – ataxia telangiectasia mutated serine/threonine protein kinase

ATR – ataxia telangiectasia and Rad3 related serine/threonine protein kinase

BAC – bacterial artificial chromosome

BCR – B cell receptor

BRCT – BRCA1 binding domain

CDK – cyclin dependent kinase

Chr – chromosome

CSR – class switch recombination

DDR – DNA damage response

D-loop – displacement loop

DNA – deoxyribonucleic acid

DNA- PKcs – DNA protein kinase, catalytic subunit

DSB – double strand break

FHA – forkhead binding domain

H2AX – histone H2A variant X

HJ – Holliday junction

HR – homologous recombination

Ig – immunoglobulin locus

IgH – immunoglobulin heavy chain locus

IgL – immunoglobulin light chain locus

IR – ionizing radiation

Lig 3 – DNA ligase III

Lig 4 – DNA ligase IV

MDC1 – mediator of DNA damage checkpoint 1

Mre11 – meiotic recombination element 11

MRN – Mre11/ Rad50/ NBS1

NBS – Nijmegen breakage syndrome

NBSLD – Nijmegen breakage syndrome like disorder

Nbs1 – Nijmegen breakage syndrome 1 or fibrin

NHEJ – non homologous end joining

NHL – Non-Hodgkin Lymphoma

Pvt1 – plasmacytoma variant translocation locus 1

RAG – Recombination activating gene

SHM – somatic hypermutation

SKY – spectral karyotyping

SSB – single strand break

UNG – uracil DNA glycosylase

V(D)J – somatic recombination of Variable, Diverse, and Joining gene segment

Abstract

Many B cell lymphomas are driven by translocations between a proto-oncogene and the B cell specific immunoglobulin heavy chain (IgH) gene. These translocations are thought to be the result of aberrant class switch recombination (CSR), a developmental process in B cells that requires programmed DNA double strand breaks (DSBs). The Mre11/Rad50/Nbs1 (MRN) complex plays multiple roles in DNA repair including sensing and binding DSBs, tethering and processing broken ends, and signaling to downstream repair pathways. To explore role for MRN in the translocation process, we engineered mice that harbor Mre11 deficiencies in B lymphocytes. These mice exhibit an 80% defect in CSR, with a significant accumulation of unrepaired DNA breaks in IgH. These unrepaired breaks are capable of participating in translocations but, surprisingly, mice do not succumb to B cell lymphoma. Thus, it is possible that MRN has crucial roles in tumor formation.

Following translocation, the proto-oncogene must become deregulated in order to confer growth advantage to a cell. Regulatory elements within IgH are known to be required for this deregulation; however the regulatory role of distal cis-acting elements in deregulation has not been examined. To determine if there are elements outside of IgH that are responsible for this, we utilize a transgenic mouse model in which a transgene containing the entire IgH locus is inserted into different chromosomal locations in different mouse lines. We observe that the transgene is able to undergo oncogenic translocations at multiple chromosomal sites and in

multiple orientations with respect to the endogenous locus. Sequencing reveals that these translocations are similar to translocations to endogenous IgH.

These results suggest that Mre11 plays multiple roles in DNA repair during B lymphocyte development, which has implications for the formation of lymphomagenic translocations. Additionally, the larger chromosomal environment, outside of the 230kb IgH locus, plays only a minor role in translocation and subsequent deregulation of an oncogene.