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Role of MAGE proteins in the stability of genome

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ROLE OF MAGE PROTEINS IN THE STABILITY OF GENOME

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ROLE OF MAGE PROTEINS IN THE STABILITY OF GENOME

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Abstract

All living organisms is made up of DNA; a molecule that contains information needed for organism's development and function. This molecule codes set of instructions known as genes which are organised into various structures called Chromosomes. The entire of chromosome structure makes up the genome which must be transmitted through generations without integrity comprise for life to continue in its original make up. The genome is reliable to several modifications and damages following normal physiological activities and other factors capable of causing physical and chemical changes in the structure of the DNA. Genome make up also includes complex cellular signal machinery involved in recognition of DNA damages, signalling/recruitment of repair factors, inducing specific repair pathway depending on the type of damage and finally the repair of the DNA damage. These series of activities by cellular signal complex ensures the maintenance of genomic integrity. Recently, a new set of protein called MAGEs has been found to be involved in the DNA damage signal complex machinery. These proteins are known to be expressed in testis for its normal physiological development but the therapeutic challenges of cancer has led researchers into discovery the involvement of MAGEs in maintaining intact genome of cancer cells. MAGE proteins ability to hamper cancer treatment through several mechanisms that centred on association and altering DNA damage repair proteins has been linked to being a predecessor of an important protein involved in chromatin structure organization and replication; the Structural Maintenance of Chromosome (SMC). In this review, the detailed important of SMC in genome maintenance is discussed as well as its relationship with MAGEs as ancestral MAGE. The role of SMC and MAGE in maintenance of genome both during replication and repair of DNA damages after replication is discussed.

Keywords: SMC, MAGE, DDR

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LIST OF ABBREVIATIONS

AMPK	AMP activated protein Kinase
ATM	Ataxia Telangiectasia Mutated
ATR	Ataxia telangiectasia and Rad3 related
BARD1	BRC-associated RING domain 1
BER	Base Excision Repair
BRC1	Breast Cancer type 1
CETN2	Centrin 2
CHK1	Checkpoint Kinase 1
CTA	Cancer Testis Antigen
CSA	Cockayne Syndrome WD repeat Protein A
CSB	Cockayne Syndrome Protein B
CtIP	CtBP-Interacting Protein
DDR	DNA Damage Response
DNA	Deoxyribo Nucleic Acid
DNA-PKcS	DNA-dependent protein kinase catalytic subunit
DSB	Double Strand Break
FA	Fanconi Anaemia
FAAP24	Fanconi Anaemia Associated Protein 24
FBP1	Fructose-1,6-biphosphate
GG-NER	Global Genome Nucleotide Excision Repair
MAGE	Melanoma Antigen Gene
MHD	MAGE Homology Domain
MMR	Mismatch Repair
HAWK	HEAT proteins associated with kleisin
H2AX	Histone 2A member X
HDAC	Histone Deacetylase
HECT	Homologous to E6-associated protein C-terminus
HLA	Human leukocytes antigen
HMGN1	High Mobility Group Nuclesome-binding domain-containing Protein 1
HR	Homologous Recombination
ICL	Interstrand Cross-link
IDLS	Insertion-deletion loops
KAP1	KRAB domain Associated protein 1
KITE	Kleisin Interacting tandem winged helix Element
KRAB-ZFPs	Kruppel Associated Box Zinc Finger Proteins
MFH	Histone Fold Protein
MHC 1	Major Histocompatibility Complex 1
MRL	MAGE-RING Ligases
MRN	MREII/RAD50/NSB1
MSH	Muts homolog/heterodimer
NDN	Necdin
NER	Nucleotide Excision Repair
NHEJ	Non-Homologous End Joining
NSE	Non-SMC Element
PARP	Poly ADP Ribose Polymerase
PCNA	Proliferating Cell Nuclear Antigen
PI3K	Phosphatidylinositol 3-kinase
PIKK	Phosphoinositide 3-kinase-like Kinases
RAD23B	Radiation Sensitive 23B
RING	Really Interesting New Gene
ROS	Reactive Oxygen Species
RPA	Replication Protein A

SAM	S-adenosyl Methionine
SMC	Structural Maintenance of Chromosome
SSB	Single Strand Break
TADs	Topologically Associated domains
TC-NER	Transcription Coupled Nucleotide Excison Reapir
TFIIH	Transcrition Initiation Factor II H
TIF1B	Transcriptional Intermediary Factor 1 beta
TLS	Translesion Synthesis
TRIM28	Tripartite Motif-containing 28
USP7	Ubiquitin Specific-Processing Protein 7
UVSSA	UV-stimulated Scaffold Protein A
VDJ	Variable, Diversity and Junctions
XLF	XRCC4-like Protein
XPC	Xeriderma Pigmentosum Complemention group C
XRCC4	X-ray Repair Cross-Complementing protein 4

Introduction

1.0 DNA Damages and Repair

The existence of all living organisms including human is dependent on the core molecules of life; the DNA and its transmission through generations without compromising its integrity. This is equally important to cell lines. Nuclear genome which consist of DNA molecules is organized in linear chromosome in eukaryotes[1]. In order to maintain the integrity of genetic information and transmit to the offspring, every damage to the DNA has to be repaired with no or minimal errors[2]. Genomic material is maintained by a complex signalling system that is capable of detecting DNA damages. Accumulation of unrepair damages can be lethal to the cells. The cellular machinery is also capable of arresting cell cycle until repair is complete[3]. This complex signalling system involves identification of DNA damages, recruitment of specific repair factors and activation of repair factors as well as selection of the right repair pathway[4] depending on the type of damage.
DNA damage is defined as "Any modification in the physical and/or chemical structure of DNA resulting in an altered DNA molecule which is different from the original DNA molecule with regard to its physical, chemical and/or structural properties"[5]. Change in DNA structure due to damages can either be in form of new structures that does not naturally exist in DNA molecule or appearances of structures (such as triple stranded DNA) at ectopic sites. DNA damages are caused by toxic agents of various sources commonly known as genotoxic[5].

1.1 Types of DNA damages

There are several kinds of damages that occur in the genome, these damages can result from endogenous and exogenous sources. Endogenous DNA damages result from hydrolytic and oxidative reactions between self-cleavage DNA and water (hydrolytic reaction) or self-cleavage DNA and reactive oxygen species within cells[4]. Endogenous damages are due to alteration during normal physiological cycle of DNA (replication errors, bases modification like spontaneous base deamination, depurination (hydrolytic cleavage of β-N-glycosidic bond of deoxyadenosine or deoxyguanosine), hydrolysis of phosphodiester groups, alkylation, oxidation of bases etc) and cellular metabolism that induces reactive oxygen species (ROS). Exogenous factors are of physical or chemical sources such as ultra-violet radiation, ionizing radiation, cigarette smoke, X-rays, alkylating agents, polymerase inhibitors, topoisomerase inhibitors, aromatic amines, toxins and environmental stress[2,4]. Example of exogeneous damage are pyrimidine dimers, intrastand and interstrand cross-links, single strand and double strand breaks of phosphodiester bond[4].

1.1.1 Hydrolytic Deamination of DNA Bases: Hydrolytic Base Deamination occurs when bases loose the exocyclic amine groups. Such of these nucleotides are cytosine, adenine, guanine and 5-methylcytosine which hydrolysis into uracil, hypoxanthine, xanthine and thymine respectively[4]. Amongst these nucleotides, cytosine and 5-methyl cytosine are the most frequently deaminated nucleotides. Environmental effect on the DNA such as intercalating agents, radiation, sodium bisulfite can promote the rate of base deamination[4].

1.1.2 Oxidation of Bases: ROS such as superoxide radicals, hydrogen peroxide and hydroxyl radical are known to have negative impact on DNA when in high level within the cell[5]. Examples of effect of ROS interaction with DNA includes; increasing double bonds of DNA bases (hydroxyl radical attack on C5/C6 double bonds of thymine produces thymine glycol) removal of hydrogen atoms from the methyl groups of DNA bases, hydrolysing the sugar molecule of DNA and exposure of DNA backbone to generate single strands break[4].

Purine and pyrimidine bases are affected by ROS to form a different molecules such as 8-oxopurines oxidized form of purine or thymine/uracil glycol oxidized form of pyrimidine[5]. The formation of these new molecules can lead to strand breaks. DNA damage by oxidation is linked to a major source of disease and ageing[5].

1.1.3 Hydrolysis of Bases (base loss): Hydrolysis is a reaction that leads to loss of nitrogenous bases. DNA depurination and depyrimidination are products of DNA base hydrolysis. Depurination spontaneously occur $5x10^3$ - $1x10^4$ times per day per genome[5]. Temperature is the major determinant of rate of DNA depurination and depyrimidination, high temperature increases the rate of base hydrolysis[5].

1.1.4 Alkylation: Alkylation can arise due to endogenous or exogenous factors. Endogenous sources is likely to be a resultant effect of exposure to metabolic intermediate such as S-adenosyl methionine(SAM). Alkylation results in DNA damage via addition of reactive alkyl groups to specific atoms of nucleophilic bases such as nitrogen 7 of guanine and nitrogen 3 of adenine[4]. The formation of 7-methylguanine (7mG) and 3-methyladenine causes alteration in base pairing and obstruct replication.

1.1.5 Pyrimidine dimers: Dimerization of DNA bases are the resultant effects of electromagnetic radiation. Ultraviolet light are responsible for dimer formation. Dimers can occur among bases of same strand or between bases of another strand[5]. Pyrimidine dimers may either produce 6-4 photoproducts or cyclobutene, both are molecule formed from pyrimidine dimerization[5].

1.1.6 Base mismatches: Mismatching of bases is a resultant effect of base deamination or base conversions and it is one of the critical DNA damage as it can cause alteration of DNA sequence[5]. Base conversion is the change of base from its original form to another due to modification e.g methylation of cytosine on position 5 in the heterocyclic ring produces 5-methylcytosine. Uracil formed from deamination of cytosine can change base pair of C:G to U:A thus creating a substitution of C:G pair with A:T pair. Same with adenine, hypoxanthine from adenine changes the base pair of A:T to A:C pairs, guanine deaminated to xanthine pairs with thymine thus substituting C:G to A:T[5].

1.1.7 Intrastrand and Interstrand cross-links: DNA lesion resulting from crosslinking agents causing bases of complementary strands to covalently link together. Example of such cross-linkers includes alkylating agents, platinum compounds, formaldehyde, psoralens etc[4]. Aldehyde in the proximity of chromatin can produce Schiff base through series of reaction, the product react with amine of another DNA base to produce intrastrand/interstrand cross-links).

1.1.8 Bridges between DNA and proteins: This bridge between DNA and proteins is otherwise known as DNA-protein crosslinks (DPCs) is formed by covalent linkage between nucleotide residue and protein. Sources of DPCs can be from different sources either natural or synthetic compounds of environment, therapeutic and endogenous origin. Examples of DPC inducing agents includes; environmental agents (ultraviolet rays, ionization radiation, metals), therapeutic agents (camptothecin, etoposides, 5-aza-2'-deoxycitidine), endogenous agents (non-enzymatic: reactive aldehydes, and enzymatic: topoisomerase inhibitor)

1.1.9 Single-stranded and double-stranded breaks of the ribose-phosphate skeleton: Single-strand breaks are one of the common DNA damages in living cells and it occurs as a result of break of phosphodiester bonds of the DNA backbone. Phosphodiester

bond break can result during nuclear activities (replication, transcription etc) and cell metabolism (oxidative damage from accumulated ROS)[5].

Double-strand breaks (DSB) is referred to the type of DNA lesion that damage simultaneously the two complementary strands due to break in the phosphodiester bond of both strands of DNA[5,6]. DSB in the genome are less frequent than other kinds of DNA damages in living cells and result from either endogenous or exogenous factors[2–4] comprising of physical, chemical and biological sources[6]. DSB is the most severe DNA lesions[7]. DBS is produced during normal process of some types of cells for example, during meiosis, DSB is essential for DNA segments exchange between non-sister chromatids of homologous chromosomes. Also DSB is required for innate immune system development process[5] such as different combination of variables, diversity and junction (VDJ) sequences after specific DSB at segment to obtain immunoglobulins[2].

DSBs can generate translocations, deletions, breakage and fusion of chromosomes[5]. Unrepaired DSB can induce apoptosis and senescence[6]. The summary of all DNA lesions and appropriate pathways involved is presented in figure 1.

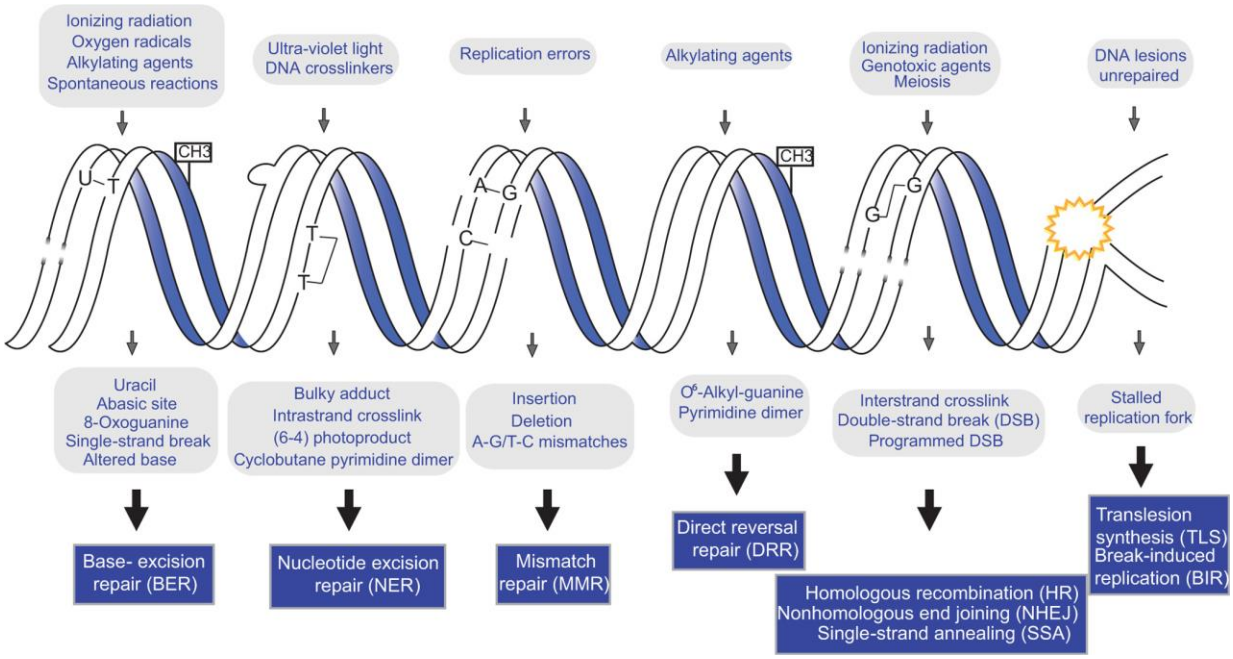

Figure 1: DNA Damage and the Associated DNA Repair Pathways.
The DNA backbone can be attacked by several endogenous or exogenous agents (for instance, ionizing radiation, alkylating agents, or oxygen radicals), leading to the activation of DNA repair enzymes. Adopted from[8].

1.2 DNA Repair Pathways

To preserve genetic integrity, cells have evolved complex signal system called DNA damage response (DDR) to identify and initiate appropriate repair mechanisms. DNA damage response is a signal transduction pathways triggered by different forms of DNA lesions of which different sets of proteins are involved[9].

Several DNA-repair mechanisms are responsible for maintaining genome integrity at both cellular and organism level. These repair pathways includes Mismatch repair (MMR), Nucleotide Excision repair (NER), Base Excision repair (BER), Homologous Recombination (HR) and Non-Homologous End Joining (NHEJ)[10].

The NER, BER and MMR mechanisms are mainly involved correcting DNA damages that affects single nucleotide or nitrogenous bases. Damages to nucleotides repair involves splicing out damaged parts and replaces the gap with new bases[11].

1.2.1 Base Excision Repair (BER): BER processes are involved in correcting certain types of DNA damages such as oxidative DNA damage, base deamination, alkylation and abasic site single base damage. The major proteins of BER pathway are enzymes known as DNA glycosylases of either monofunctional or bifunctional[4,8]. The former has only glycosylase function while the latter in addition to glycosylase function possess apurinic/apyrimidinic (AP) lyase activity capable of converting base lesion to single-strand break thus making it possible for DNA ligase to ligate the nick ends[8]. During cell cycle, BER is mainly active in the G1 phase where it recognizes and excised damaged base from DNA helix. BER is known to contribute to the global maintenance of genome stability as it equally occurs in mitochondria[4].

1.2.2 Nucleotide Excision Repair (NER): NER is responsible for excising bulky lesions caused by UV radiation or chemotherapeutic agents[4]. NER possesses a complex repair machinery called nucleotide excision repairosome which consists of a large number of different proteins. It is responsible for removing damaged region of DNA of about 30 nucleotide length via generation of bimodal incisions in flanking region[11]. NER are of two major types; global genome NER (GG-NER) and transcription-coupled NER (TC-NER)[4,8]. GG-NER is responsible for scanning the entire genome for base lesions independent of transcription. TC-NER recognizes lesions that obstructs the transcription machinery[8]. The pathway repairs DNA damages in three steps; DNA damage recognition, DNA helix unwinding and incision, DNA repair synthesis and ligation[8]. In GG-NER, a DNA damage sensor (complex) called Xeroderma Pigmentosum complementation group C (XPC) and Radiation sensitive 23B (RAD23B); XPC-RAD23B complex with Centrin 2 (CETN 2) recognizes and binds UV-radiation induced lesions. The detection and binding of XPC-RAD23B/CETN 2 to UV-radiation induced lesions are assisted by UV-damaged DNA-binding protein complex which consist of DDB1 and GG-NER-specific protein; DDB2. XPC possess a binding domain for a multifunctional factor known as transcription initiation factor ll H (TFllH) complex, which function as transcription initiation and repair factor. TFIIH recognizes XPC-RAD23B/CETN 2 at damage site and then bind to its domain on XPC to coordinate (at 5' and 3' end of the lesion) and gap filling activity.
In TC-NER pathway, TC-NER specific proteins; Cockayne Syndrome WD repeat protein A (CSA) and Cockayne Syndrome protein B (CSB) are recruited by lesion stalled RNA polymerase which in turn recruits other TC-NER components such as high mobility group nucleosome-binding domain-containing protein 1 (HMGN1), ubiquitin specific-processing protein 7 (USP7), UV-stimulated scaffold protein A (UVSSA) at the lesion site. After recruitment and assembling of all factors and components of TC-NER is completed, CSA-CSB complex reversely translocate RNA polymerase 11 to expose damage region which initiates TFllH recruitment for repair and transcription initiation[4].

1.2.3 Mismatch Repair (MMR): Considering the basal level of pairing error (1 in 10^9 to 10^{10} base pairs) in cell division, there arise a need for post replicative repair in order to increase DNA replication fidelity up to 1000-fold. This responsibility of increasing replication fidelity is done by MMR pathway[8]. MMR occur in post-replication repair, homologous recombination, class-switch recombination[4]. MMR equally involves in homologous recombination where it ensures that strand exchange between nonhomologous sequence does not occur[8]. Base mismatch and insertion-deletion loops (IDLs) are substrates of MMR pathway. The three major processes of MMR is recognition, excision and DNA synthesis[8]. MMR gene are evolutionary conserved[8]. Protein responsible for recognition of base mismatches and IDLs in eukaryotes are MutS homolog (MSH) polypeptides. Human possess eight of this polypeptides[4]. Base mismatches and short IDLs are recognized by MutSα heterodimer (MSH2/MSH6) while large IDLs are sensed by MutSβ heterodimer (MSH2/MSH3). MutLα heterodimer (MLH1/PMS2 heterodimer) equally support the activity

of MutS while the initiation step is mediated by DNA polymerases processivity factor proliferating cell nuclear antigen (PCNA)[4].

1.2.4 Repair of Inter-strand lesions (Interstrand cross-link repair, ICL): ICL results in covalently linked double strands and causes bidirectional stall in the progression of replication fork[2]. To overcome the barrier created by ICL, three major processes are required which includes incision, TLS polymerases activity and recombination process[2]. Important proteins of Interstrand cross-link repair pathway are Fanconi Anaemia (FA) proteins consisting of 21 functional groups (A, B, C, D1, D2, E, F, G, I, J, L, M, N, O, P, Q, R, S, T, U, V). FANCM recognizes and assemble at ICL lesions and recruits Fanconi Anaemia Associated protein 24KDa (FAAP24) and histone fold protein complex (MFH). FANCM and MFH are known to stimulate remodelling of replication fork to promote Holliday junction migration and creates single strand DNA gaps[4]. Single strand DNA is then bound by replication protein A (RPA) to trigger activation of ATR which phosphorylate CHK1. Phosphorylated CHK1 in turn phosphorylate FANCE, FANCD2, FANCI and MRN[4]. Phosphorylated FANCI-FANCD2 complex and other core members of the FA groups localizes at the ICL site and FANCL (an ubiquitin ligase) mono-ubiquinate FANCI-FANCD2 complex to activate FA pathway[2,4]. Though different steps are involved as regards to either replicating cell or non-replicating cells but the general concept after the activation of FA pathway remain same. Endonucleases responsible for lesion excision on the DNA strand at 5' and 3' end are activated resulting in the release of the lagging strand from the crosslink. In the next step, TLS polymerases uses the leading strand harbouring the ICL as a template for new DNA synthesis while NER pathway eliminates the ICL from the leading strand after bypass of TLS polymerases[4].

1.2.5 Trans-lesion synthesis (TLS): TLS is one of the main DDR pathways' effector that is imperative for recovery of DNA replication at stalled replication forks[12]. It involves low fidelity TLS DNA polymerases called "sloppy copiers" to bypass DNA lesions such as thymine dimers[11]. TLS polymerases can synthesize new DNA to fill in gaps created by repair machinery[4]. TLS involves a switch from DNA replication polymerases to TLS DNA polymerase capable of tolerating large lesions. TLS activation and lesion bypass requires mono-ubiquitination of PCNA). An E3 ubiquitin ligase, RAD18 is known to be responsible for specific mono-ubiquitination of PCNA at residue K164. The mono-ubiquitinated K164 facilitates the interaction between PCNA and TLS polymerase at stalled replication site. In the absence of TLS, DNA double strands break can occur due to a persistent S-phase arrest by unresolved checkpoint kinase signals[12]. An illustration of TLS process is shown in figure 2.

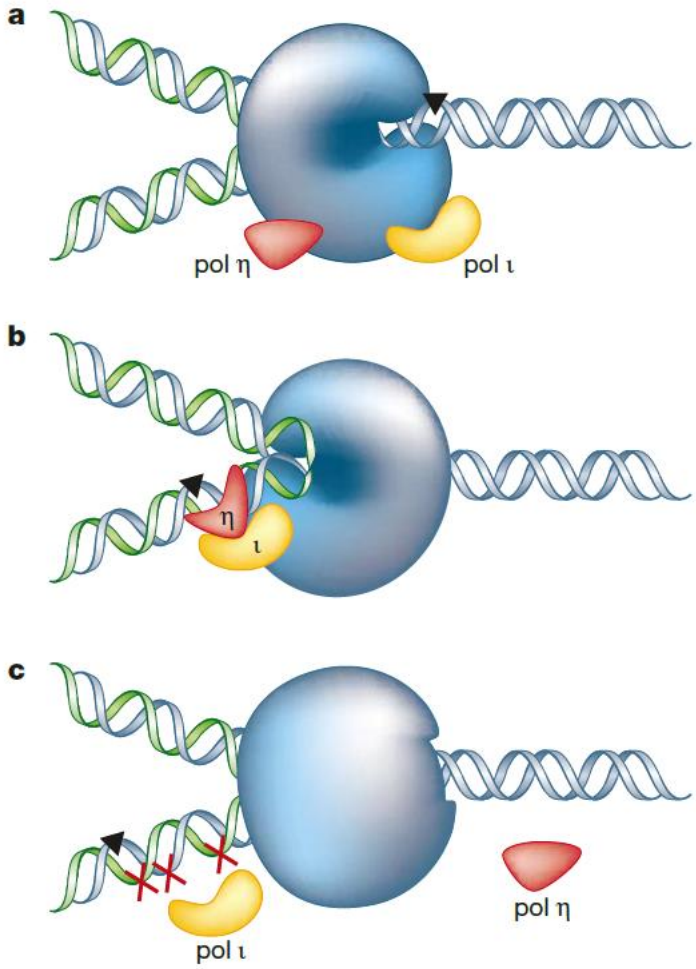

Figure 2: 'Sloppy copiers' overcome blocks in replication caused by a DNA lesion (a process called trans-lesion synthesis). **a**, The DNA replicative machinery (blue) stalls immediately behind a site of base damage (black triangle). Two specialized 'sloppy copier' polymerases (polh and poli) bind to the arrested replication complex. **b**, This interaction promotes a conformational change in the arrested replication machinery, placing polh in direct proximity to the site of base damage where it synthesizes across the lesion. **c**, Polh may then dissociate and allow poli to complete the process of replicative bypass by incorporating several more nucleotides (red crosses). Once the lesion has been completely bypassed, the replication machinery resumes DNA replication. As a result of this process, mutations to the DNA sequence are now incorporated into one strand[11].

1.2.6 DSB Repair: Two members of phosphoinositide 3-kinase-like kinases (PIKK), Ataxia Telangiectasia Mutated (ATM) and Ataxia Telangiectasia and Rad3 related (ATR) are key determinants of DDR signalling network activation when DSB occur and are equally important for regulation of HR-mediated repair. Either SSB or DSB repair is dependent on the PIKK family that is activated. As a general rule, the transducers ATM and ATR are activated for DSB and SSB break repair respectively[9]. Survival of eukaryotic cells is highly dependent on efficient repair of DSBs by either of the DSB repair pathway; homologous recombination (HR) or non-homologous end joining (NHEJ) repair[4].
In eukaryotes, DSB are sensed and immediately targeted by MRE11/RAD50/NBS1 (MRN) complex which is a scaffold for ATM recruitment[4,10] also to process DSB ends making them available for RPA binding[4].

1.2.6.1 Non homologous end joining (NHEJ): NHEJ is an error-prone repair mechanism that involves ends joining of broken DNA strands without considering sequence homology. It can cause nucleotides loss and chromosomal translocation[13]. Though the effect of repair errors is minimal as large parts of mammalian genomes are non-coding[8]. In a cell cycle, NHEJ is responsible for repair of damages that occur during the G1, G0 and M phases[13]. A

number of proteins plays a vital role in this pathway. Upon recognition of DSBs, Ku (Ku70 and Ku80) heterodimer quickly binds DSBs to avoid ends degradation and form the basis for the assembling of NHEJ components[4]. The Ku70/Ku80 protein is responsible for recruiting DNA-dependent protein kinase catalytic subunit (DNA-PKcs) to the break sites which initiates NHEJ[8].
Next step after DNA-PKcs assembling is the recruitment of XRCC4/LIG4 in complex with stimulatory factor XLF for ligation of broken ends[2].

1.2.6.2 Homologous recombination (HR): HR is an error-free repair pathway involving use of sister homologous chromatid as a template for the repair of the broken strands[13]. The choice of sister chromatid as a template is thought to be favoured as a result of sequence identity, spatial alignment and physical cohesion of the chromatids[4]. HR occurs during S and G2 phases of the cell cycle[4,13]. It is a multistep process of DSB repair linked to risk of cancer due to the involvement of two hereditary cancer predisposition genes known as BRCA1 and BRCA2[4]. The knowledge of these genes involvement has led to development of poly(ADP-ribose) polymerase (PARP) inhibitors as a therapeutic strategy for BRCA-linked cancers. Defects in HR showed sensitivity to PARP inhibitors[4].
HR begins with formation of nucleoprotein filament by recombinase RAD51 on single strand DNA[4,13] targeting gaps on post-replicative strand or extended 3' ssDNA tail generated end processing nuclease activity[4]. PARP is thought to initiate the HR pathway. PARP binding to DSB instead of Ku heterodimer proteins initiates the assembling of a sensor protein: MRN which stimulates the recruitment of ATM at the DSB site. ATM is responsible for phosphorylating DDR mediators such as H2AX, 53BP1, MDC1 and BRCA1[2].
RPA binding stimulates the interaction of CtBP-interacting protein (CtIP)[4].
Breast cancer type 1 (BRCA1) protein form complex with BRCA1-associated RING domain 1 (BARD1) protein[4]. BRCA1-BARD1 complex interaction with CtIP and MRN is important for DNA end resection. BRCA2 is a recombination mediators that facilitate RAD51 nucleoprotein filament formation[4].

2.0 SMC5/6 Complex: Structure and Function

Structural Maintenance of Chromosome (SMC) complexes are proteins responsible for higher-order organization of chromatin and genome replication[14]. There is a similarity between prokaryotic and eukaryotic complex in terms of core composition and architecture[14].
SMC proteins are of three cores protein complexes[15] which perform various functions in chromatin organization[14]. Studies have described three SMC complexes in prokaryotes (SMC/ScpAB, MukBEF, MksBEF) and three SMC complexes in eukaryotes (cohesin, condensin and SMC5/6)[16]. These complexes are composed of two SMC proteins of long coiled-coil structure and non-SMC, subunits known as KITE (Kleisin interacting tandem winged helix element) in prokaryotes/eukaryotes SMC5/6 and HAWK (HEAT proteins associated with kleisin) in condensin and cohesin and kleisin proteins that serve as a connector of the long coiled-coil molecule head[14]. The KITE and HAWK proteins are non-SMC subunits known as non-SMC elements (NSE)[17]. Studies first identified SMC5/6 complex in budding yeast (Saccharomyces cerevisiae) as a complex with six NSE (Nse1, Mms21/Nse2, Nse3, Nse4/Qri2, Nse5, and Kre29/Nse6) subunits and fission yeast (Saccharomyces pombe) as complex with four NSE (Nse1, Nse2 (Mms21), Nse3 (Ydr288W), and Nse4 (Qri2)) subunits[18,19]. Human orthologs of the fission yeast (Saccharomyces pombe) has been identified which are named as hSmc5, hSmc6, hNse1, hNse2/hMms21, hNse3/MAGE1, and hNse4 and they equally formed complex in vivo[15]. SMC proteins contains a central helical region called hinge which extends at both ends to amino terminal (Walker A motif) and carboxyl terminal (Walker B motif). The hinge domain enabled the folding back of the walker domains to form an ATPase head domain[15,19]. An overview of the structure of SMC complexes is presented in figure 3.

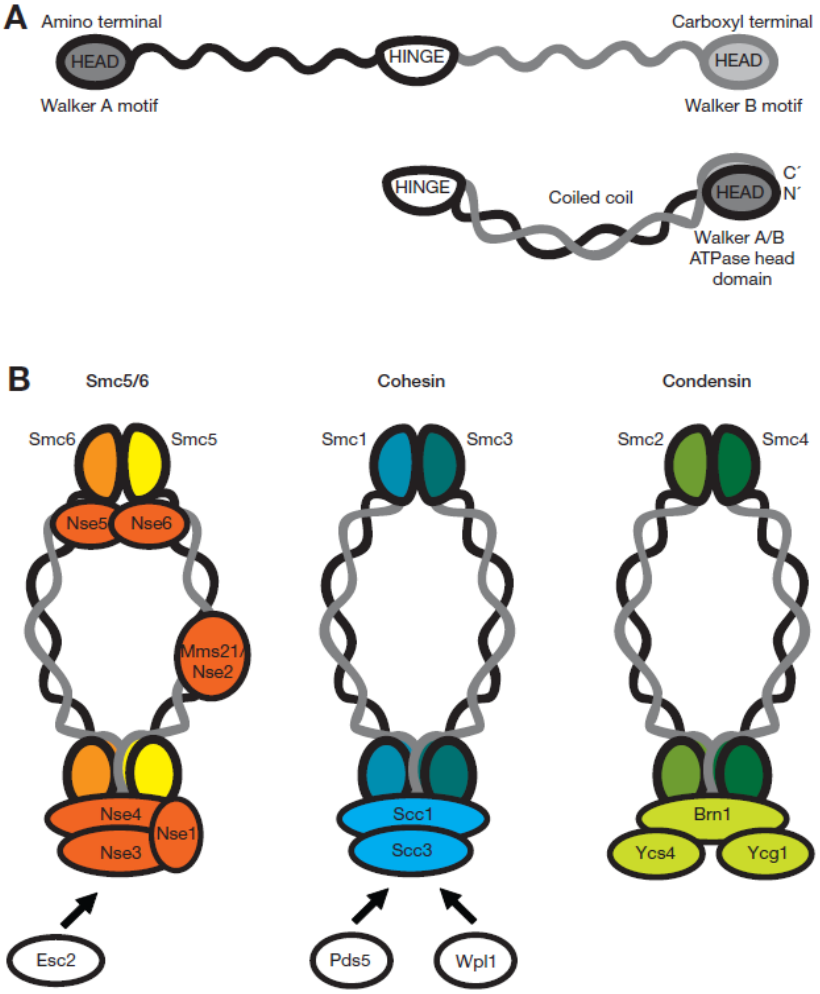

Figure 3. Structure and composition of SMC complexes.
(*A*) An SMC protein is folded at the central hinge domain. This brings the Walker motifs in the amino- and carboxy-terminal globular domains together, creating an ATPase that is connected to the hinge domain via a long stretched coiled coil. (*B*) General organization of Smc5/6, cohesin, and condensin. Subunits are named according to the budding yeast nomenclature. With the exception of the ATPase activity of SMC proteins, only Smc5/6 contains a protein with enzymatic activity, the Nse2/Mms21 SUMO ligase. Adopted from [19].

In S. pombe, only four of Nse subunit proteins of SMC5/6 are essential for survival while in S. cerevisiae, all six Nse are important for proliferation. Nse1 possesses a RING finger domain similar to those found in E3 ubiquitin ligase but there is not yet an evidence of similar activity. Nse2 is a sumo ligase for SMC5 in S. cerevisiae but a sumo ligase for SMC6 in S. pombe. Nse3 is a member of the MAGE family of proteins while Nse4 belongs to the kleisin superfamily[15]. SMC6, SMC5 and Nse4 were first identified as Rad18, Spr18 and Rad62 respectively[19]. Study have shown that Nse1 RING-like domain is important for assembling of NSE1-NSE3-NSE4 in a sub-complex involved in DDR[1].
In eukaryotes, SMC complexes are key regulators of chromosomes structure, dynamics and functions[1]. Cohesin (SMC1/3) and condensin (SMC2/4) functions are documented as being essential for sister chromatid cohesion/chromosome alignment in mitosis and for condensation of chromosome/DNA damage repair respectively[15,17]. The precise function of SMC5/6 complex has not yet been fully established but thought to be involve in resolving DNA damage and recombination structures[15]. The initial function of SMC5/6 complex was first deduced from yeast mutant SMC5/6 being hypersensitive to UV light, ionizing irradiation, camptothecin, and other genotoxic agents as methyl methanesulfonate, hydroxyurea e.t.c[20]. Characterization of human and mouse SMC5 and SMC6 proteins showed interaction of the two proteins which is mediated by a hinge region. Mutation study revealed the hinge to contain a conserved glycine which is crucial for its role as a molecule connector.

Taylor et al made an interesting revelation of mouse SMC5 (mSMC5) and SMC6 (mSMC6), their expression was found in all tissues but was localized to the sex vesicle during meiosis[15]. SMC5/6 complex performs distinct functions crucial for genome integrity maintenance[21]. Some of these functions includes restarting of collapsed replication forks, regulation of chromosomal topology, maintenance of heterochromatin including ribosomal DNA, chromosome replication, telomeres maintenance, homologous recombination of damaged somatic cell's DNA and meiotic recombination[14,21].

The discovery of SMC6 mutant (also known as RAD18-X mutant) in yeast being sensitive to ultraviolet, infrared, chemical agents and alteration in replication has led to more study of SMC5/6 complex involvement in DNA damage repair. Study has shown that following DSB in yeast during G2/M, SMC5/6 and cohesin are recruited in a RAD50/MRE11 dependent way thus activating HR pathway. In human the recruitment of SMC5/6 is dependent on RAD18 and SLF1/SLF2 dimer (an ortholog of the yeast Nse5/Nse6 dimer) activity, of HR pathway.

The ring structure of cohesion and SMC5/6 helps to hold and align DNA strands in order to promote HR. The promotion of HR pathway is due to the ability of SMC5/6 and cohesin to hold and align two DNA double helix in their ring structures.

SMC5/6 is shown to play important role in sister chromatid recombination. It is believed that Nse2 might be partly responsible for sumoylation of cohesin Scc1 subunit which promotes sister chromatid recombination.

2.1 SMC and Stressed Replication fork: SMC5/6 is involved in recovery of replication fork. Study revealed that mutant SMC5/6 in S. cerevisiae equally harbour mutations in the genes responsible for promoting stalled replication fork recovery such as srs2, sgs1/rqh1 thus an evidence of SMC5/6 role in stalled replication fork recovery[14]. Collapse of RF and or unloaded replisome result in formation of X-shaped joint molecules which requires HR pathway for their resolution. It has been shown that SMC5/6 recognizes and binds to recombination intermediate molecules formed when replication fork collapse and with activation of its helicase activity via sumolylation resolves the intermediates joints that blocks replication progress[14]. The SUMO-dependent intervention of SMC5/6 has equally shown to restrain replication fork regression and stabilizes stalled replication fork especially when DNA is enriched in repeats leading to replication-fork-pausing site are being transcribed thereby preventing accumulation of recombinant intermediates. Examples of such molecules includes rDNA, transfer DNA (tDNA), centromeres and telomere[14].

2.2 SMC Promote Normal Progression of Replication

Research revealed enhanced chromosomal association of SMC5/6 which is suggested to be based on transient structure formed during replication. The structures includes super helical tension of DNA helix and sister chromatid intertwines. Though these structures can be resolved by topoisomerase II after replication, they can inhibit sister chromatid segregation causing prolonged G2/M arrest. SMC5/6 ensures that these structures are resolved for replication to continue without prolonged cell cycle arrest[14].

Depletion of SMC5/6 results in aberrant chromosome phenotype and segregation as well as abnormal localization of topoisomerase IIα and condesin in mitosis[14].

3.0 MAGEs and their Classification

The first Melanoma AntiGene Encoding gene (MAGE) was first discovered in early 1990s and was named MAGE-1[21,22]. MAGE-1 was from a melanoma cell line called MZ2-MEL, a cell line derived from a stage IV melanoma cancer patient who for a decade had relapse of the cancer (despite multiple surgery) and strong cytotoxic T-cell activity against the tumour[21,22]. MAGE proteins are one of the families of Cancer-Testis Antigen (CTA) proteins (types of proteins strictly expressed in germline cells, trophoblasts for some of them and cancer cells). Cancer antigens are peptides of approximately 10 amino acids generated from cancer cells by peptidases and presented on major histocompatibility complex class 1 (MHC 1) molecule to

cytotoxic T lymphocytes for adaptive immune response[23]. Other members of CTA includes members of GAGE and XAGE families[24]. MAGE-1 is part of a group of tumour-associated antigens that share a conserved amino acid domain referred to as MAGE homologous domain (MHD)[25–31]. MAGE family proteins consist of more than 40 proteins that are expressed primarily in cells of the germ line and placenta. Human MAGE family proteins are generally classified based on their expression pattern in tissues, sequence homology and chromosome location. The classification includes type I and type II classes[21,22,28]. Type I comprises the MAGE-A, MAGE-B and MAGE-C subfamilies[22,28]. The type I MAGE genes are located as clusters on X chromosome and are strictly express in germ cells of the testis and for some of them in the placenta. They exhibit high degree of homology among themselves as well as co-expression in cancers[21,22,28]. Type I MAGEs are known for their aberrant expression in many cancer cells (melanoma, pancreatic cancer, breast cancer, colon, brain, lung, prostate, non-small cell lung cancer and ovarian cancer as well as hematopoietic) and have been identified as cancer biomarkers[21,25–27,30–33]. Among type I MAGE, studies have linked MAGE-A and MAGE-C2 expression to aggressive progression, higher stage, treatment resistance and worst clinical prognostic of carcinomas[28,30,34].

Type II MAGE genes consist of MAGE-D, MAGE-E, MAGE-F, MAGE-G1, MAGE-H, MAGE-L and Necdin (NDN) of which some are located on X chromosome (MAGE-D, MAGE-E, and MAGE-H) while others, MAGE-L2, MAGE-G1, NDN and MAGE-F1 are on autosomes. These genes are expressed in many normal tissues and do not encode antigenic peptides[21,22,28]. An overview of the MAGE proteins classification in human and mouse is presented in figure 4.

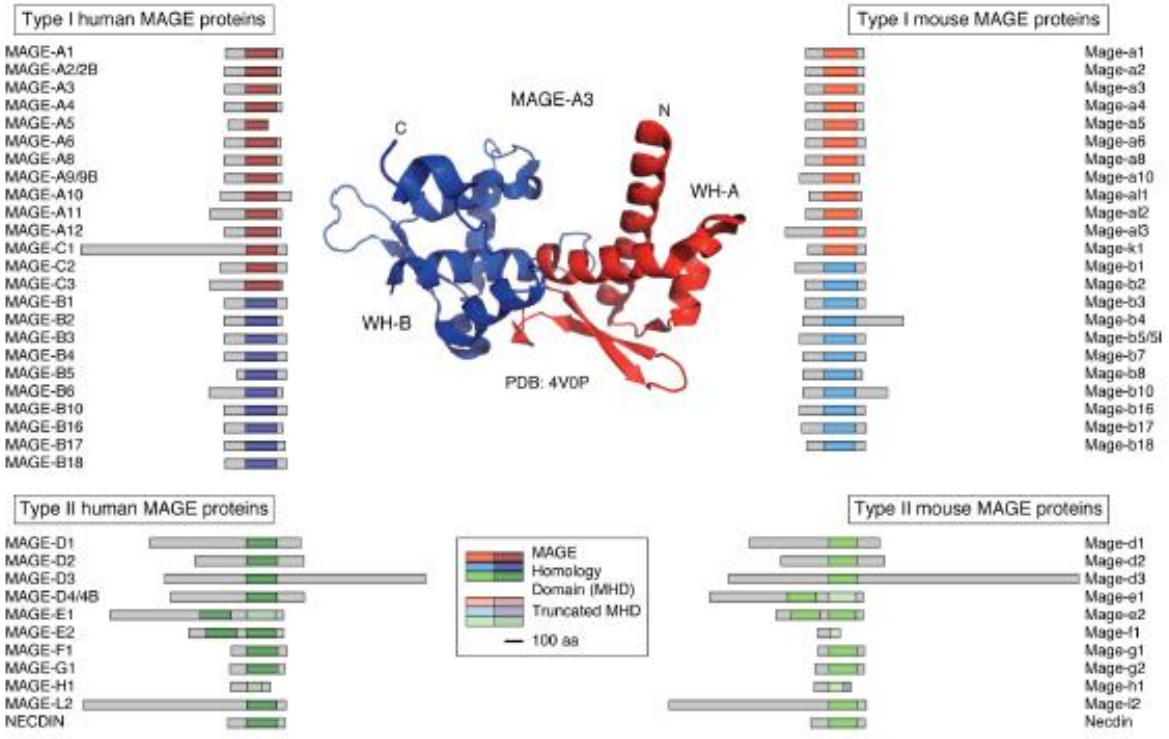

Figure 4: Representation of Human and Mouse Classes of MAGE Proteins. Adapted from[21].

In type I MAGEs, the subfamilies -A, -B and -C are encoded by a single exon[35]. MAGE-A genes are located in Xq28, MAGE-B genes in Xp22 and MAGE-C in Xq26-27[21]. Human MAGE-A comprises of 12 genes coding for 11 proteins (MAGE-A7 is a pseudogene) and they share between 50% and 96% of identity (MAGE-A3 and -A6 differ only by 14/314 aa). Therefore, functional redundancy between some of these proteins can be expected. MAGE B consist of 10 genes while MAGE-C comprise of 7 genes[28,36]. Majority of type II MAGEs are composed of single exon but MAGE-D genes contain 13 exons with 11 being coding[35].

3.1 Evolution of MAGE

MAGE genes are believed to evolve from non-SMC element 3 (Nse3), a subunit of SMC5-6 protein complex[37]. MAGE can be traced back to protozoans where it existed as a single copy gene[35]. Most eukaryotes have a single MAGE gene, including non-mammalian vertebrates such as chicken, frogs and fish showing that the expansion of the MAGE family occurred during eutherian radiation. The MAGE-D genes share the gene structure (13 exons) of the single MAGE found in non-eutherian vertebrates. All other mammalian genes have been generated by retro-transposition and duplication events[21]. Taylor et al discovered that MAGE-G1 is the functional homologue of Nse3 in the SMC5/6 complex. hNSE1 and hSMC6 were used to immunoprecipitated hSMC5/6 complex. Mass spectroscopy was performed on the complexes and the result obtained as shown in table 1 reveal that MAGE-G1 is part of the complex formation[15].

TABLE 1. Mass spectrometric analysis of proteins copurifying with hNSE1 and hSMC6[a]

Protein	Purification with:			
	hNSE1		hSMC6	
	No. of peptides	% Coverage	No. of peptides	% Coverage
hSMC5	28	19.9	7	6.6
hSMC6	21	23.5	8	8.4
hNSE1	5	18.6		
hMMS21/hNSE2	5	19.0		
hNSE4a	6	17.1	2	8.1
MAGEG1	4	17.9	3	3.5

[a] Anti-hNSE1 or anti-hSMC6 antibodies coupled to protein A-Sepharose were used to immunoprecipitate the indicated proteins from HeLa nuclear extract. Eluted complexes were analyzed by mass spectrometry. The number of independent peptides of each protein recovered and the percentage of primary sequences covered by these peptides are presented.

Surprisingly, the function of NSE3 in eutherians is performed by a retrogene (MAGE-G1) rather than by direct descendant of NSE3 which is MAGE-D2.

Evolutionary history of MAGE gene family has been proposed to be in four phases. Phase 1 described the existence of ancestral MAGE as a single copy gene in protist until the divergence of monotremes, marsupials and eutherians[35,38]. Evidence is related to the existence of a single MAGE homolog in monotremes[38]. In phase ll, radiation study in eutherians is suggested to have triggered the emergence of MAGE subfamily through retro-transposition of MAGE genes except for MAGE-C which is thought to result from MAGE-A duplication. Phase lll is characterized by duplication within the subfamilies. Phase 4, human MAGE-A genes through sequence divergence generated different epitopes encoded by human leukocytes antigen (HLA) class 1molecule[38]. An illustration of the this evolution is presented in figure 4.

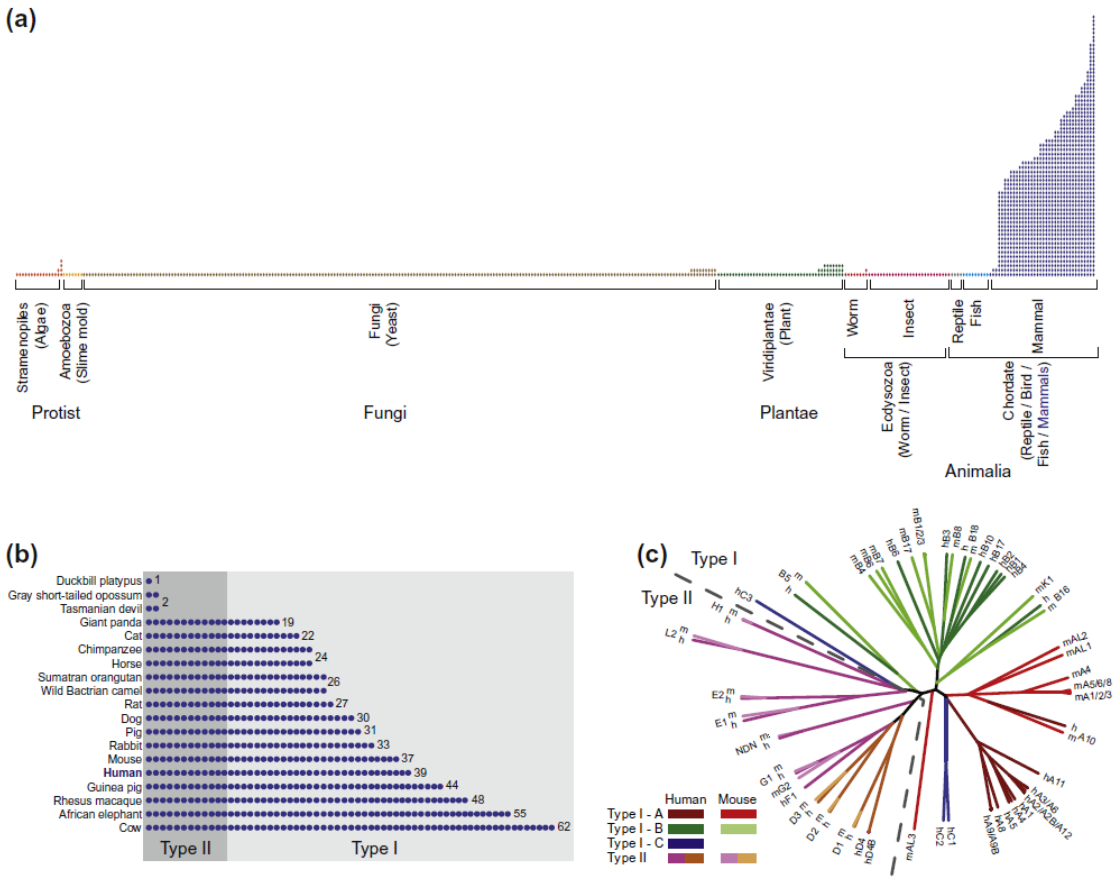

Figure. 5. Evolution of the MAGE genes. (a) The MAGE family is evolutionarily conserved in all eukaryotes. Following the emergence of eutherian mammals (blue), the MAGE family underwent a rapid and dramatic expansion from a single MAGE in lower eukaryotes to a large multigene family. Each column represents an organism with the number of circles denoting the number of MAGE proteins in each organism based on pfam annotation. (b) A detailed view of the recent expansion of MAGEs in select mammals. The type II MAGEs (designated based on the human MAGEs) are more evolutionarily ancient, while the type I MAGEs appear to be the result of recent gene duplications. (c) The type II MAGEs share high homology with their mouse orthologs, whereas type I MAGEs share much higher sequence conservation within their respective subfamilies compared to their mouse orthologs. Adopted from[35].

3.2 Molecular Functions of MAGE

Efforts have been made to characterized the molecular functions of MAGE and one of the most studied function is MAGE interaction with ubiquin ligases.

Ubiquitination is a post-translational modification that regulate several cellular processes such as DNA repair, signal transduction and protein degradation[39]. Ubiquitination selective degradation of protein by 26S proteasome is mediated by conserved lysine residues[39,40]. Ubiquitin (Ub) is a highly conserved polypeptide of 76 residues that can be found in all types of cells and contains 7 lysine residues namely K6, K11, K27, K29, K33, K48 and K63[40]. Three important enzymes are involved in ubiquitination, they include ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2) and ubiquitin ligase (E3)[39,40].Ubiquitination process involves 3 steps with each of the ubiquitin enzymes playing a specific role. In the first step, E1 activate ubiquitin molecule in an ATP-dependent manner to form a thioester bond between the Ub carboxyl group and the thiol of active E1 residue (cysteine). Afterwards, the cysteine residue of E2 enzyme receive the activated ubiquitin for onward passage to E3 ligase. E3 ligase is responsible for attaching ubiquitin moieties to lysine residue on targeted protein[40]. Ubiquitination involves cellular signals which is dependent on precise substrate, E3 is crucial for substrate selection thus the possible explanation for their sensitivity[39]. Based on various protein-protein interaction manner, E3 ligases are grouped as Homologous to E6-associated Protein C-terminus (HECT), Really Interesting New Gene (RING) and U-box Domain[40].

Study have shown that RING type through its docking sites (RING domain) interact with MAGEs and form MAGE-RING ligases (MRLs)[21].
Studies showed that MAGE (specifically MAGE-A and MAGE-C2) proteins are capable of forming complex with specific E3 RING ubiquitin ligases (TRIM28/KAP1, TRIM31, TRIM69 and TRIM27) *in vivo* and this binding is specific to each MAGE[21,34]. MAGE proteins have been shown to bind RING E3 ligases through MHD. MHD motif is an intrinsically disordered domain; a characteristic that is important in MAGE-RING ligase complex formation to bring about a regulatory function for specific substrate[21].This specific binding of E3 ubiquitin ligases to MAGE has been proven to alter the ubiquitination process and activities of E3 ligases as well as their subcellular localization[34]. The major activity of MAGE is to enhance ubiquitination and degradation of target proteins.
Several biological functions of MAGE proteins have been described.
For instance MAGE-D1 (NRAGE) is known to interact with the p75 neurotrophin receptor and regulate apoptosis during neural development. Necdin is expressed in neurons and regulates proliferation, survival and differentiation[41].
MAGE-G1 (hNSE3) is a unit of the SMC5/6 complex responsible for proliferation and chromatin dynamics regulation during DDR[41].

3.3 MAGE in Genomic Stability and DDR

The several repair mechanisms of DNA damages are triggered by DDR depending on the type of lesion that occur[2]. Some of the processes involves in DDR are cell-cycle arrest, apoptosis and senescence which are the results of p53-dependent regulatory pathway. These processes involves the CDK inhibitor p21, pro-apoptotic BAX and PUMA proteins[2]. An important protein of DDR pathway is KRAB domain-associated protein 1 (KAP1), otherwise known as tripartite motif-containing 28 (TRIM28) or transcriptional Intermediary factor 1 beta (TIF1B). KAP1 is a widely expressed nuclear scaffolding protein and E3 ubiquitin ligase of approximately 106kDa. KAP1 possess at its amino terminal a special domain called RING-B box coiled coil for which it interacts with KRAB protein. The domain serves as a binding site for MAGE MHD[28,31,42]. KAP1 regulates several physiological activities such as cell differentiation, DDR, immunological response and tumorigenesis by interacting with Kruppel-associated box zinc finger proteins (KRAB-ZFPs) mostly as transcriptional co-repressor[43]. KAP1 possess in addition to its RBCC domain, other domain which forms complexes with a large number of chromatin remodelling proteins (HP1, HDAC, HMTs, DNMTs, CHD3/NuRD), affecting epigenetic regulation of many genes[43]. KAP1 equally possess transcription-independent functions linked to cell cycle status and chromatin complexity. Proteins of the PIKK family during DDR phosphorylate KAP1 at serine 824 to initiate association with DDR factors (such as γH2AX, 53BP1, TOPBP1) and stimulates ATM-mediated chromatin relaxation[43,44]. KAP1 is capable of regulating transcription factors lacking repression domain such as c-Myc and E2F1. The level of KAP1 repressive function is regulated by multiple post-translational modification[44].
MAGE-D1 (NRAGE) also regulates DDR pathway especially homologous recombination via interaction with RNF8 and BARD1. RNF8 and BARD1 are involved in DDR via regulation of H2AX ubiquitination for signal transduction. MAGE-D1 interacts with these proteins via their RING domain to stabilize their complex and facilitate DDR signal transduction[45]. A study showed a role for NRAGE in DDR. NRAGE depleted cells showed increased expression of ɣH2AX suggesting a role of NRAGE in cancer resistance to induced genotoxic stress. A DNA repair reporter assay revealed a 10-fold more efficient HR in cells overexpressing NRAGE compared to NRAGE depleted cells whereas NHEJ repair was not impacted. An *in-vitro* and *in-vivo* study to determine the effect of a chemotherapeutic agent on NRAGE depleted cancer cells in nude mice. Cancer cells showed their sensitivity by low survival percentage and low tumour growth[45].
MAGE-D2 has been described as a nucleolus protein involved in DDR. In normal conditions MAGE-D2 is found in cytoplasm, nucleoplasm and nucleoli. In response to DNA damage,

transition from Gap 1 to synthesis phase is blocked to activate repair mechanisms. MAGE-D2 is then excluded from nucleoli and concentrate in the nucleoplasm upon DNA damage where it is phosphorylated by ATM/ATR. It can equally negatively regulate p53 transcriptional activity[21].

MAGE-G1 (NSE3) form part of the SMC5/6 complex which is involved in HR repair of DSB to maintain genomic stability. A study proves that NSE3 is crucial for SMC5/6 complex. The study assesses the effect of induced genotoxic stress in cell line deficient in SMC5/6 complex. Sensitivity to induced genotoxic stress following MAGE-G1 knockdown was observed[21].

3.4 Type 1 MAGEs and DDR

Aside the core DDR factors and pro-apoptotic proteins, MAGEs are important proteins involved in DDR in cancer cells and usually in germ cells. In response to DNA damage, a complex containing KAP1 condenses and represses chromatin. Phosphorylated KAP1 (pKAP1-s824) accumulates at the sites of DSBs maintain the activities of ATM thereby promoting DNA damage repair [36,46]. When DSB occurs, KAP1 condenses chromatin and induces gene repression by recruiting histone proteins and enzymes involved in chromatin compaction[31]. KAP1 response to DSB is mediated by zinc finger transcription factors (ZNF) bound to KRAB domain[31]. MAGE-A3 and C2 stabilizes KAP1 to KRAB domain zinc finger transcription factor[41]. MAGE-C2 has also been suggested to improve response of DDR proteins to cellular endogenous or induced stress by increasing KAP1 Ser824 phosphorylation by ATM upon interaction with KAP1[36]. The complex formations between MAGE and KAP1 have impact in a number of physiological functions such as p53 and AMPKα1 signalling[21]. MAGE-A (A2, A3 and A6) and MAGE-C2 have the ability to promote genome integrity via DSB repair by binding to KAPI through their MHD to facilitate phosphorylation of the complex by ATM. MAGE-A3 and -C2 regulate KRAB-ZFP mediated gene expression through their interaction with KAP1. MAGE-A3/-6-KAP1 complexes regulate gene repression either by promoting ubiquitination of transcription factors regulators or by directly causing repression independently of ubiquitination. MAGE-C2 through binding to a E3 ubiquitin ligase RING domain protein Rbx1 inhibit cyclin E ubiquitination causing its abundance to promote G1-S transition thus cell proliferation. DSB repair is equally promoted by MAGE-C2-KAP1 complex through increase in KAP1 Ser-824 phosphorylation by ATM[21]. MAGE-A1 and A2 regulates transcription through targeting of histone deacetylase (HDAC) activity.

3.5 MAGE-A4 and TLS

MAGE-A4 is implicated in promoting TLS process. The di-leucine motif of MHD is believed to induce catalytic activities of this cognate E3-ligases. Interestingly, in MAGE-A4, this motif stabilizes its cognate partner; RAD18-RAD6 complex which promote PCNA mono-ubiquitination and TLS[12]. MAGE-A4 involvement in TLS was studied by Yanzhe Gao et al. MAGE-A4 promotes DNA damage repair during replication by positively influencing RAD18 and TLS function in cancer cells. Contrary to the known phenomenon of MAGEs activating E3 ligase, MAGE-A4 associate with RAD18 in order to stabilize it for stimulating PCNA mono-ubiquitination thus triggering TLS. This shows that MAGEs, aside inducing E3-ligase catalytic function, equally affects the signal transduction of E3 through stabilization[12].

3.6 MAGE and p53

Type I MAGEs have been mainly investigated in cancer cells where they have been proposed to drive tumorigenesis by many ways and importantly by regulating KAP1[21,34]. MAGE-A3,-A6 and -C2 reprogram KAP1 to ubiquinate p53 and two major metabolic proteins; AMPK and FBP1[21]. p53 is a transcription factor that maintains genome integrity by suppressing cell proliferation through four important mechanism; (1) induction of cell cycle arrest during

stalled replication fork, (2) initiating apoptosis of unrepairable and damage cells, (3) induction of senescence and (4) DNA repair[47]. p53 is regulated and stabilized through KAP1-KRAB complex[21]. p53 functions can be impaired by MAGE proteins different mechanisms. MAGE form complex with KAP1 and promote p53 degradation. In the context of melanoma, repression of p53 expression and function is achieved principally by DNA deacetylation. MAGE-A achieves this regulation by binding to p53 and recruiting of HDAC3 to promoter binding sites of p53 thus switching its activating function to certain gene such as p21 to repressive function. MAGE-A proteins play a crucial role in survival and proliferation of multiple myeloma cells through regulation of anti-apoptotic mechanisms[33]. MAGE-A proteins also bind to the DNA binding domain of p53 and interfere with its transcriptional activity[21]. Anti-apoptotic activity of MAGE-A proteins is basically observed for MAGE-A2,-A3, -A5, -A6 an -C2[48]. Study by Yang *et al* showed that type I MAGEs (MAGE-A2, -A3, -A5, -A6 and –C2) enhance and stabilize KAP1/p53 complex formation and act as molecular cofactors for KAP1-dependent p53 suppression. MAGE-A enhance p53 ubiquitination and subsequent proteasomal-mediated degradation via interaction with KAP1[30,33]. Another study showed that pro-apoptotic proteins (Bax, caspases-3,-7 and -9) were upregulated following silencing of MAGE-A9 expression resulting in de-ubiquitination of p53 and stabilization of p53 to increase the expression of pro-apoptotic proteins[49]. Another apoptosis regulation of p53 activity by MAGE-A is mediated by MDM2 (an E3 ubiquitin ligase)[30]. MAGE-A also prevent regulatory activity of p53 responsive genes by association with DNA-binding site of p53[21].

4.0 Conclusion

Genome instability induces different pattern of evolution, non-maintenance of intact genome, cancer development, aging and other human diseases[4] Well-coordinated DNA repair process is crucial in preventing several pathological conditions especially cancer[2]. Stability of genome is dependent on the mechanisms involves in replication, segregation and repair of damaged chromosomes. The replication, segregation and repair processes requires intricate protein complexes[16,19]. A member of SMC complexes family; SMC5/6 is essential for chromosome maintenance and specifically involves in chromosome segregation and repair mechanisms[16]. "Cohesins hold newly replicated sister chromatids together, promote sister chromatid recombination, and are responsible for the dynamic organization of chromatin fibres during interphase into topologically associated domains (TADs). Condensins play key roles in the compaction and individualization of chromatids in mitosis while SMC5/6 complexes are implicated in the repair of DNA damage by homologous recombination, stabilization and restarting of stressed RFs[14]. SMC6 has been implicated in assisting Rad 52 to recover stalled replication fork at rDNA repeats[50]. SMC5/6 subunits is known to possess enzymatic activity in its Nse1 (ubiquitin ligase) and Nse2 (SUMO ligase)[51]. Although SMC5/6 has no helicase or cleavage activity, it plays a major role in sumoylating other DDR proteins[50]. Mutations in Nse3 disrupt SMC5/6 leading to instability of the complex and its inability to participate in DDR[50]. SMC directly participates in DDR pathway through its ATPase dependent-complex member; RAD50[2]. Cancer therapeutic strategy has been hampered with the involvement of MAGE proteins as it helps cancer cells to repair targeted DNA damage. MAGE interaction with KAP1 has been shown to enhance p53 ubiquitination via proteasome–dependent pathway[30]. Equally KAP1 is a co-repressor of p53, study have shown MAGE type I family members form complex with KAP1 thereby regulating p53 apoptosis-independent regulation[52].

MAGE-A4 targets RAD18 for genome maintenance and stability in cancer cells via stabilizing RAD18 for increased PCNA mono-ubiquitination and TLS activation. In tumorigenesis, RAD18-TLS pathway has been implicated in maintaining genome viability during oncogene-induced replication stresses via promotion of error-prone DNA synthesis[12].

MAGE-C promote tumour development by its association with KAP1. DNA repair and cellular proliferation are promoted following heterochromatin relaxation facilitated by KAP1-Ser824 phosphorylation[53]. Study by Bhatia et at al suggest that MAGEs induces ATM-dependent phosphorylation of KAPI-Ser824 to promote DDR in cancer cells post-treatment with genotoxic. Since resistance to chemotherapy has been associated with MAGE expression[53], it is therefore important to explore mechanism by which this association and all consequences it causes to other DDR factors can be hampered to improve cancer therapy.

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