



IRIBHM

Faculty of Medicine

**CELLULAR AND MOLECULAR MECHANISMS UNDERLYING THE
MAINTENANCE OF GENOMIC INTEGRITY IN EPIDERMAL STEM
CELLS**

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PhD academic degree in Biomedical Sciences

Academic year 2012-2013

Acknowledgements

Cette toute première page s'adresse à tous ceux qui ont contribué, de (très !) près ou de (plus) loin, à l'élaboration de cette thèse, depuis ses prémisses, jusqu'à ce jour.

Mes premières pensées vont vers mon directeur de thèse, Cédric Blanpain, sans qui ce travail n'aurait pu commencer ni aboutir. Je voudrais te remercier pour ton accueil au sein de ton laboratoire, de m'avoir si bien préparé à cette bourse, un challenge qui me semblait au début insurmontable. Pour ton encadrement tout au long de ces années, et de m'avoir transmis ta passion de la science. Un si grand merci d'avoir partagé avec moi cette qualité scientifique incroyable qui émane de toi.

I would like to express my warmest acknowledgements to the members of my jury for their time to judge this work.

In this very specific chapter, there is one person I cannot overlook. My deepest acknowledgements go to Dr Panagiota Sotiropoulou, a.k.a Peggy. Before you, I had many more defaults (not speaking at all about my legendary organization of the first year, where I could start a BrdU flow kit at 2 pm...). It is from you that I learnt the most during these last four years. I always enjoyed working with you as well as discussing science, Brussels, Greece, fashion, ... I will never forget the team we were, you and me. You even taught me some Greek words! I hope that this thesis will be an "Epliktiki epithia"! Again Peggy, all my deepest acknowledgements for everything.

Un tout grand merci à tous ceux et celles qui ont partagé avec moi ces (longues) journées qui ont jalonné ces années. Merci à Khalil pour ta bonne humeur, ta sélection musical, Guilhem, Giuseppe, Gaëlle Lapouge, Andrea, Virginie, Cindy, Samira, Marielle, Karine, Antoine, Alexandra, Ben B (merci pour tous ces papiers que tu m'as filés !), Ben D (courage dans les files venant de Gand, je compatis !), Greg, Maud (merci pour ta patience quand j'essayais de te transmettre l'amour pour mon métier, merci), Sophie (merci pour ce si beau bouquet de fleur que tu as organisé encore !) Amélie, Gaëlle B, etc. Un tout grand merci pour tout ce que vous m'avez tous apporté. Un tout grand merci à ceux qui ont pu participer à notre journée de mariage, cela nous a fait très plaisir!

Un grand merci à Jean-Marie Vanderwinden pour ta patience à me transmettre tes conseils et connaissances de la microscopie.

Un grand merci également à Jean-Christophe Marine et son équipe pour ses conseils et son aide précieuse dans la réalisation de ces fameux WB du « DNA repair ». Combien de temps avons-nous passé Peggy à « scraper » et « sortir » pour une petite bande ;-) ??

Mes remerciements se tournent également vers mes parents qui ont su m'encourager dans les moments difficiles et qui se sont efforcés à me voir persévérer dans mon domaine. Même si un de vous regarde maintenant tout cet agitement d'en haut. Je remercie également ma belle famille pour tous leurs encouragements tout au long de ces longs mois.

Merci à toute l'équipe « Oncology » de ThromboGenics qui m'a chaleureusement accueilli.

Ce dernier paragraphe, comme la dernière étoile dans le « Hall of Fame » revient à mon mari, David, qui a toujours été là à mes côtés me soutenant, me comprenant (mes langages codés de biologiste ;-)), me rappelant parfois (souvent !) gentiment quand je cours « comme une poule sans tête ». C'est aussi grâce à ce courage et cette motivation que tu m'as apporté tout au long de ces années qu'on y est quoi !

Ce travail a pu être réalisé grâce au soutien du FRIA-FNRS.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS.....	I
TABLE OF CONTENTS	III
ABBREVIATIONS.....	VI
CHAPTER 1 - SUMMARY	1
CHAPTER 2 - INTRODUCTION	3
2.1 THE MURINE SKIN EPIDERMIS: AN OVERVIEW	3
2.1.1 <i>Morphogenesis and maintenance of the skin inter-follicular epidermis</i>	3
2.1.2 <i>Development and cycling of hair follicle</i>	4
2.1.3 <i>Different types of SCs in the murine skin epidermis</i>	5
2.1.3.1 Multipotent Adult Bulge Stem Cells	5
2.1.3.2 Stem cells of the inter-follicular epidermis (IFE).....	7
2.1.3.3 Isthmus and sebaceous gland stem cells	7
2.2 THE DNA DAMAGE RESPONSE AND REPAIR	8
2.2.1 <i>Sources of DNA damage-inducing agents</i>	8
2.2.1.1 Endogenous sources	8
2.2.1.2 Exogenous sources	8
2.2.2 <i>Mechanisms of activation of the DDR</i>	9
2.2.2.1 Sensors and transducers.....	9
2.2.2.2 Mediators.....	10
2.2.2.3 Effectors	11
2.2.2.4 Cellular outcomes of activation of the DNA Damage Response.....	12
2.2.3 <i>Specific repair mechanisms and their associated pathologies</i>	17
2.2.3.1 Double-Strand Break repair	17
2.2.3.2 Nucleotide Excision repair (NER).....	23
2.2.3.3 Base Excision repair (BER).....	24
2.2.3.4 Mismatch Repair (MMR).....	25
2.2.3.5 Direct reversal of DNA damage.....	25
2.2.4 <i>Modes of chromosomes segregation</i>	25
CHAPTER 3 - AIM OF THE PROJECT.....	28
CHAPTER 4 - RESULTS	29
4.1 INVESTIGATING THE MODE OF DNA STRAND SEGREGATION AS A POTENT GENOME PROTECTION IN BULGE SCs	30

4.2	DNA DAMAGE RESPONSE AND REPAIR OF BULGE SCs TO IR.....	34
4.3	ROLE OF HR DURING MORPHOGENESIS OF THE SKIN EPIDERMIS.....	37
4.3.1	<i>Specific deletion of Brca1 in the skin epidermis results in congenital alopecia.....</i>	38
4.3.2	<i>The number of placodes is reduced and hair follicle formation is delayed upon Brca1 deletion.....</i>	38
4.3.3	<i>Brca1-deleted HF are fewer and fail to maintain over time.....</i>	38
4.3.4	<i>Constitutive deletion of Brca1 in the epidermis leads to loss of adult bulge SC markers and progressive exhaustion of HF progenitor markers.....</i>	39
4.3.5	<i>Interfollicular, upper isthmus and sebaceous glands are mostly unaffected by epidermis-specific Brca1 deletion.....</i>	40
4.3.6	<i>Loss of function of Brca1 in the epidermis triggers p53 stabilisation.....</i>	40
4.3.7	<i>Sustained p53 activation leads to high apoptosis and proliferation defects.....</i>	40
4.3.8	<i>Conclusions.....</i>	41
CHAPTER 5 -	DISCUSSION	46
5.1	BULGE SC LABEL-RETENTION DURING MORPHOGENESIS AND ADULTHOOD REFLECTS THEIR RELATIVE QUIESCENCE BUT NOT AN ASYMMETRIC STRAND SEGREGATION	46
5.2	MODES OF CHROMOSOME SEGREGATION IN OTHER TYPES OF SCs	48
5.3	BCL-2 FAMILY AND ACCELERATED DNA REPAIR MEDIATES RESISTANCE OF DIFFERENT TYPES OF ADULT SCs TO DSBs-INDUCED CELL DEATH	51
5.3.1	<i>DDR in human Epidermal SCs: differences in species?.....</i>	51
5.3.2	<i>DDR and repair in HSCs: are SCs of another high turnover tissue sharing genome protection mechanisms?</i>	52
5.3.3	<i>DDR and repair in ISCs and colonic SCs: also a high turnover tissue.....</i>	54
5.3.4	<i>Melanocytes SCs: the niche neighbour.....</i>	55
5.3.5	<i>DDR in mammary gland SCs</i>	56
5.3.6	<i>Conclusions.....</i>	56
5.4	THE FASTER REPAIR OF DSBs IN BULGE SCs RESULTS IN A MORE TRANSIENT P53 ACTIVATION	57
5.5	RESISTANCE TO DNA DAMAGE-INDUCED APOPTOSIS OF BULGE SCs DOES NOT RESULT FROM THE PROLIFERATIVE STATUS OR THE IR DOSE	58
5.6	GENOME PROTECTION MECHANISMS IN SCs TO OTHER TYPES OF DNA DAMAGE AGENTS.....	59
5.7	IMPLICATIONS OF GENOMIC INTEGRITY IN ADULT SCs AND ITS CONSEQUENCES IN CANCER AND AGEING.....	60
5.8	THE DIFFERENT TYPES OF EPIDERMAL SCs ARE DIFFERENTIALLY AFFECTED AFTER HR-DEFICIENCY.....	61
5.9	DIFFERENTIAL IMPORTANCE OF EACH REPAIR PATHWAY DURING DEVELOPMENT, HOMEOSTASIS AND DIFFERENTIATION.....	64
5.10	IS THE ROLE OF BRCA1 IN THE SKIN INDEPENDENT OF ITS DNA REPAIR ACTIVITIES?	66
CHAPTER 6 -	CONCLUSIONS.....	68
CHAPTER 7 -	PERSPECTIVES	70
7.1	ROLE OF miRNAs IN DDR AND REPAIR OF BULGE SCs	70

7.2	DDR AND REPAIR IN CSC OF CUTANEOUS SKIN CANCER.....	71
7.3	EFFECT OF OTHER DNA DAMAGING AGENTS ON BULGE SCs.....	72
7.4	DDR IN HF PROGENITORS AND PROLIFERATING ADULT SCs UPON IR EXPOSURE	73
7.5	DDR AND REPAIR IN OTHER EPIDERMAL SCs AND PROGENITORS	74
CHAPTER 8 - BIBLIOGRAPHY		75

ABBREVIATIONS

- 53BP1: P53 Binding Protein 1
- 6-4PPs: 6-4 photoproducts
- APC: Adenomatous Polyposis Coli
- ATM: ataxia telangiectasia, mutated
- ATR: ATM and Rad3-related
- BCC: Basal Cell Carcinoma
- Bcl-2: B-cell lymphoma 2
- Bcl-XL: B-cell lymphoma-extra large
- Brca1: breast cancer 1, early onset
- Brca2: breast cancer 2, early onset
- BRCT: BRCA1 C Terminus
- BrdU: 5-Bromo-2'-deoxyuridine
- caspases: Cisteinyl ASpartate ProteASE
- Cdk: Cyclin-dependent kinase
- CEBP α : CCAAT-enhancer binding protein- α
- Chk1: checkpoint kinases 1
- Chk2: checkpoint kinases 2
- cKO: conditional Knock-Out
- CldU: 5-Chloro-2'-deoxyuridine
- CO-FISH: Chromosome Orientation-Fluorescence In situ Hybridization
- CPDs: Cyclobutane-Pyrimidine Dimers
- CSC: Cancer Stem Cell
- Ctip: C-Terminal Binding Protein Interacting Protein
- DDR: DNA Damage Response
- Dkk1: dickkopf homolog 1
- DMBA: 7,12-dimethylbenz[α]anthracene
- DNA-PK(cs): DNA-dependent protein kinase (catalytic site)
- DP: Dermal Papilla
- DSB(s): Double-Stranded Break(s)
- DSBR: Double Strand Break Repair or Double Holliday junction model
- dsDNA: double-stranded DNA
- EPU: Epidermal Proliferative Unit
- FACS: Fluorescence Activated Cell Sorting
- Foxo3A: Forkhead box O3A
- GBM: Glioblastoma
- GOF: Gain of function
- Grhl3: Grainy head-like 3
- GSH: Glutathione
- H/E: Hematoxylin/Eosin
- HF: Hair Follicle
- HG: Hair Germ

- HIF-1 α : Hypoxia-inducible factor 1, alpha subunit
- IdU: 5-Iodo-2'-deoxyuridine
- IFE: Inter-Follicular Epidermis
- IHC: immunohistochemistry
- Inv: Involucrin
- IR: Irradiation
- IRF6: Interferon regulatory factor 6
- K1: Cytokeratin1
- K10: Cytokeratin10
- K14: Cytokeratin 14
- Lef-1: lymphoid enhancer-binding factor 1.
- LOF: Loss of function
- LOH: Loss of heterozygosity
- MAPK: Mitogen-Activated Protein Kinase
- Mcl-1: myeloid cell leukemia sequence 1
- Mre11: Meiotic recombination 11
- MRN: Mre11, Rad50 and Nbs1
- p53Aip1: p53-regulated apoptosis-inducing protein 1
- POT1: protection of telomeres 1
- PUMA: p53 upregulated modulator of apoptosis
- Rb: Retinoblastoma
- ROS: Reactive Oxygen Species
- RPA: Replication protein A
- SC: Stem Cell
- SCC: Squamous Cell Carcinoma
- SDSA: synthesis-dependent strand-annealing
- SG: Sebaceous Gland
- Smc1: Structural maintenance of chromosomes 1
- SSB(s): Single-Stranded Break(s)
- ssDNA: single-stranded DNA
- TA cells: Transient amplifying cells
- Tef: transcription factor
- TdT: Terminal deoxynucleotidyl transferase
- TPA: tetradecanoyl phorbol acetate
- TRF2: telomeric repeat-binding factor 2
- UV: Ultraviolet
- Wip1: Wild-type p53-induced phosphatase 1
- WT: Wild-Type
- XrCC4: X-ray repair complementing defective repair in Chinese hamster cells 4

Chapter 1 - SUMMARY

Adult Stem Cells (SCs) have been found in almost every organ. They are responsible for homeostasis and tissue repair after injury. SCs reside and self-renew in the adult body throughout the life of the organism. In rapid self-renewing organs, such as the skin, the intestine and the blood, SCs divide many times during the life of the animal in order to sustain the homeostatic needs of the tissue.

All cells of the body, including SCs, are constantly subjected to DNA assaults arising from endogenous sources, such as reactive oxygen species (ROS) generated by cellular metabolism, or exogenous assaults arising from the environment. The DNA damage response (DDR) and DNA repair mechanisms protect cells from accumulating DNA damage by inducing transient cell cycle arrest allowing DNA repair, triggering senescence or apoptosis.

DNA damages trigger the activation of the effectors of the DDR inducing a transient cell cycle arrest, allowing DNA repair, or triggering a permanent arrest of the cell cycle or apoptosis if damages are too extensive.

As skin is the outermost barrier of the body, epidermal cells, including SCs, are continuously subjected to genotoxic stress, such as UV rays, ionizing radiation (IR) and chemicals. The skin epidermis is composed of hair follicles (HFs), its associated sebaceous gland (SG) and the surrounding inter-follicular epidermis (IFE). Different types of SCs maintain the homeostasis of the skin; multipotent adult bulge SCs ensure the cyclic regeneration of the HF and the repair of the epidermis after injury, while individual unipotent SCs ensure homeostasis of the SG and the IFE.

In tissues with high cellular turnover, such as the epidermis, the numerous divisions that a SC undergoes could result in the accumulation of replication-associated DNA damage. It has been suggested that adult SCs may undergo asymmetric divisions in which the daughter SC retains the older (thus “immortal”) DNA strand, while the daughter cell committed to differentiation inherits the newly synthesized strand that may have incorporated replication-derived mutations. The *in vivo* relevance of this mechanism is still a matter of intense debate. We used multiple *in vivo* experimental approaches to investigate precisely how bulge SCs

segregate their chromosomes during HF morphogenesis, SC activation and skin homeostasis. Using pulse-chase experiments with two different uridine analogs together with DNA-independent chromatin labelling, we showed that multipotent HF SCs segregate their chromosomes randomly, and that the label-retention observed in the skin epidermis derives solely from relative quiescence of skin SCs ¹.

We investigated the *in vivo* response of multipotent adult HF bulge SCs to DNA damage induced by IR. We showed that bulge SCs are profoundly resistant to DNA damage-induced cell death compared to their more mature counterparts. Interestingly, we demonstrated that resistance of bulge SCs to IR-induced apoptosis does not rely on their relative quiescence. Moreover, we showed that DDR in SCs does not lead to premature senescence. We found that two intrinsic cellular mechanisms participate in the resistance of bulge SCs to DNA damage-induced cell death. Bulge SCs express higher level of the anti-apoptotic Bcl-2 and present more transient activation of p53 due to a faster DNA repair activity mediated by a non-homologous end joining (NHEJ) mechanism. Since NHEJ is not error free, this property might be a double-edged sword, supporting short-term survival of bulge SCs but impairing long-term genomic integrity ².

While we unveiled the relevance of DSBs repair by NHEJ in the skin epidermis, little is known about the role of homologous recombination (HR) during the morphogenesis of the skin epidermis. Brca1 is an essential protein for HR. Conditional deletion of Brca1 in the developing epidermis leads to congenital alopecia accompanied by a decreased density of hair placodes. The remaining HFs never produce mature hair and progressively degenerate due to high levels of apoptosis. Multipotent adult HF bulge SCs cannot be detected in adult HF in the Brca1 cKO epidermis. Brca1 deletion in the epidermis triggers p53 activation throughout the epidermis, which activates apoptosis. Interestingly, IFE and the isthmus region of the HF do not present any pathological phenotype by constitutive deletion of Brca1. Our results demonstrated the critical role of Brca1 during HF morphogenesis. Future studies will be required to understand the molecular mechanisms controlling this phenotype.