

Impact of Histone Deacetylase Inhibitors on the HPV-16
Transcriptional Activity and Genomic Integration

Thesis presented by Ekaterina Dimitrova Bojilova Albert

with a view to obtaining the PhD Degree in Pharmaceutical and Biomedical Sciences ("Docteur en Sciences Pharmaceutiques et Biomedicales") Academic year 2018-2019

Supervisor: Dr. Véronique Fontaine

Thesis jury:

Dr. Hassan Jijakli (Université libre de Bruxelles, Chair)

Dr. Stéphanie Pochet (Université libre de Bruxelles, Secretary)

Dr. Yvan de Launoit (Institut de Biologie de Lille)

Dr. Yves Poumay (Université de Namur)

Dr. René Wintjens (Université libre de Bruxelles)

Dr. Véronique Fontaine (Université libre de Bruxelles, Supervisor)

ACKNOWLEDGEMENTS:

First and foremost, I would like to thank my dynamic, resourceful, scientifically-imaginative mentor, Dr. Véronique Fontaine. Véronique has the incredible quality of absolutely always shining with positive energy, even after having worked a sixty-hour week (or weeks), which has consistently made working with her a matter of pure joy. Véronique was also the only Belgian scientist who, a long time ago, saw potential in me and gave me the chance to do a Doctorate in her laboratory. In doing that, she also gave me the opportunity to pursue my dreams.

I would also like to thank our collaborators from the Institut Pasteur de Lille, the incredible Dr. Corinne Abbadie and her senior post-Doctoral Fellow, Dr. Nathalie Martin. It is thanks to Corinne's vast knowledge and solid scientific qualities that I was able to successfully pursue a challenging project, and it is thanks to Corinne that I have (almost) managed to produce a very nice publication. As for Nathalie, thank you so much for taking away from your valuable time in order to teach me novel and exciting experimental techniques, thus considerably expanding the repertoire of my technical proficiencies.

An enormous thank you to Dr. Jean-Paul Dehaye for his limitless availability, for being a constant source of vast scientific knowledge and constructive criticism, and for his lighting-quick responses to every single one of my emails over the years. Also, all my gratitude to Naima El Manssouri, whose outstanding technical talent contributed to some of the most beautiful figures in my thesis. And not to forget, huge thanks to Drs. Rémi Rosière and Vincent Levet for the invaluable help with preparing chemicals for my experiments, with a punctuality that I find simply extraordinary.

Also, an enormous thank-you to the Amis des Instituts Pasteur à Bruxelles, for funding my Doctoral studies in the course of two years. I hope to make you proud! And, thanks to all the members of my thesis defense Jury, for their invaluable input in ameliorating my thesis, their incisive questions, and in general for agreeing to be there!

Last, but not least, I would like to thank Dr. Véronique Megalizzi, not only for all her assistance in my work, but also for being the beautiful person that she is and for offering me her friendship.

Table of Contents

ABLE OF ABBREVIATIONS	Error! Bookmark not defined.
NTRODUCTION	Error! Bookmark not defined.
I. HUMAN PAPILLOMAVIRUS	Error! Bookmark not defined.
I.1 Human Papillomavirus Life Cycle in Cervical Epithelial Ce	${ m lls}$ Error! Bookmark not defined.
I.2 Human Papillomavirus and Cancer	Error! Bookmark not defined.
I.2 (i) Cervical Cancer	Error! Bookmark not defined.
I.3 High-Risk Human Papillomavirus and Cellular Transformat	ion. Error! Bookmark not defined.
I.4 Regulation of Human Papillomavirus Early Genes Expression	on Error! Bookmark not defined.
I.4 (i) Regulation of the Viral LCR at the Transcriptional Level	Error! Bookmark not defined.
I.4 (ii) Regulation of the Viral LCR at the Epigenetic Level	Error! Bookmark not defined.
I.5 Epigenetic Regulation of Cellular Gene Promoters by HR-H Bookmark not defined.	IPV Oncoproteins Error!
I.6 High-Risk Human Papillomavirus Integration into the Geno Bookmark not defined.	me of the Host Keratinocyte Error!
II. DNA DAMAGE REPAIR	Error! Bookmark not defined.
II.1 Base Excision Repair and Single-Strand Break Repair	Error! Bookmark not defined.
II.2 Double-Strand Break Repair	Error! Bookmark not defined.
II.2 (i) Non-Homologous End Joining	Error! Bookmark not defined.
II.2 (ii) Homologous Recombination Repair	Error! Bookmark not defined.
II.2 (iii) DNA Replication Fork Stalling and Collapse	Error! Bookmark not defined.
III. CHROMATIN	Error! Bookmark not defined.
III.1 Modification of DNA and Histones by Methylation	Error! Bookmark not defined.
III.1 (i) DNA Methylation	Error! Bookmark not defined.
III.1 (ii) Histone Methylation	Error! Bookmark not defined.
III.1 (iii) Interdependence between DNA and Histone Methylat	ion. Error! Bookmark not defined.
III.2 Modification of Histones and Other Cellular Proteins by A defined.	acetylation Error! Bookmark not
III.2 (i) Histone Acetylases	Error! Bookmark not defined.
III.2 (ii) Histone Deacetylases	Error! Bookmark not defined.
III.3 Chromatin Remodelers	Error! Bookmark not defined.
III.4 DNA Damage Repair and Chromatin	Error! Bookmark not defined.

IV. HISTONE DEACETYLASE INHIBITORS	Error! Bookmark not defined.
IV.1 Mechanisms of Histone Deacetylase Inhibitor Action in Cadefined.	ancer CellsError! Bookmark not
IV.1 (i) Histone Deacetylase Inhibitor-Induced Cell Cycle Arres	st Error! Bookmark not defined.
IV.1 (ii) Histone Deacetylase Inhibitor-Induced Apoptosis	Error! Bookmark not defined.
IV.1 (iii) Effects of Histone Deacetylase Inhibitors on Cellular S Bookmark not defined.	Signaling Pathways Error!
IV.2 Histone Deacetylase Inhibitors in DNA damage signaling a not defined.	and DNA repair. Error! Bookmark
IV.2 (i) Acetylation and DNA Damage Signaling	Error! Bookmark not defined.
IV.2 (ii) Acetylation and Non-Homologous End Joining	Error! Bookmark not defined.
IV.2 (iii) Acetylation and Homologous Recombination	Error! Bookmark not defined.
IV.2 (iv) Acetylation and Base Excision Repair/Single-Strand B defined.	reak Repair. Error! Bookmark not
IV.3 Summary: Acetylation, Histone Deacetylase Inhibitors and Bookmark not defined.	DNA Repair in Cancer Error!
V. HUMAN IMMUNODEFICIENCY VIRUS-1 AND HISTON INHIBITORS	
VI. HUMAN PAPILLOMAVIRUS AND HISTONE DEACET Bookmark not defined.	YLASE INHIBITORS Error!
VII. DNA DAMAGE REPAIR AND THE HUMAN PAPILLO Bookmark not defined.	MAVIRUS LIFE CYCLEError!
OBJECTIVES	Error! Bookmark not defined.
MATERIALS AND METHODS	Error! Bookmark not defined.
Plasmids and plasmid DNA purification	Error! Bookmark not defined.
Drugs	Error! Bookmark not defined.
Cell culture and transfection	Error! Bookmark not defined.
MTT cell toxicity assays	Error! Bookmark not defined.
Measurement of luciferase activity	Error! Bookmark not defined.
Chromatin Immunoprecipitation and qPCR Analysis	Error! Bookmark not defined.
Assessment of plasmid genomic integration by stable transfect	ion Error! Bookmark not defined.
Assessment of plasmid genomic integration by qPCR	Error! Bookmark not defined.
Construction of HaCaT stable cell lines	Error! Bookmark not defined.
Characterization of HaCaT stable cell lines: genomic DNA pre Bookmark not defined.	eparation and PCR Error!
Comet assay	Error! Bookmark not defined.
Immunolabeling for fluorescence microscopy	Error! Bookmark not defined.

Preparation of cell lysates and immunoblotting	Error! Bookmark not defined.
Statistics	Error! Bookmark not defined.
ESULTS AND DISCUSSION	Error! Bookmark not defined.
RESULTS I: PRELIMINARY STUDIES	Error! Bookmark not defined.
I.1 Cellular Toxicity Studies of Common HDACi and HDACi A defined.	nalogues Error! Bookmark not
I.2 Sonication time optimization for the shearing of HaCaT and S Bookmark not defined.	SiHa cell chromatin Error!
I.3 Evaluation of primer pairs for the amplification of HPV-16 L containing AP-1 binding sites.	
II.1 Histone Deacetylase Inhibitors (HDACi) induce the HPV-16 transcriptional activity in transformed human cell lines	
II.2 The effect on HPV-16 LCR induction by the HDACi TSA is	•
II.3 Time-dependent effect of TSA on HPV-16 LCR-driven lucit is independent of viral early gene expression.	•
II.4 TSA induces HaCaT cell differentiation that can be inhibited at the time of treatment.	•
II.5 Time-dependent effects of TSA analogues on the extrachron transcriptional activity.	
II.6 The HPV-16 LCR transcriptional response to TSA treatment AP-1 binding sites.	_
II.7 TSA treatment has no effect on the transcriptional activity of Bookmark not defined.	f the AP-1 promoter Error!
RESULTS III:	Error! Bookmark not defined.
III.1 Effects of TSA and TSA analogues on the HPV-16 LCR tragenomically integrated form.	•
III.2 TSA treatment increases chromatin acetylation and methyla AP-1 binding site within the integrated HPV-16 LCR	
III.3 The presence of HPV genes modulates the epigenetic respo containing an AP-1 binding site, to TSA treatment.	
IV.1 TSA promotes the integration of plasmid DNA into the Had Bookmark not defined.	CaT cell host genome Error!
IV.2 Promoting the integration of transfected pDNA sequences i general attribute of HDACi.	_
IV.3 Construction of HaCaT cell lines stably expressing pWtLC	
DISCUSSSION IV:	Frror! Bookmark not defined.

RESULTS V: MOLECULAR MECHANISMS BEHIND THE HDACi-PROMOTED
INTEGRATION OF FOREIGN DNA INTO CELLULAR GENOMES Error! Bookmark not defined.
V.1 Treatment of HaCaT cells with HDACi causes DNA damageError! Bookmark not defined.
V.2 DNA damage in keratinocytes can be a direct consequence of HDACi treatment Error! Bookmark not defined.
Error! Bookmark not defined.
V.3 DNA Repair in HDACi-treated keratinocytes is globally compromised Error! Bookmark not defined.
V.4 Deficient DNA repair in cisplatin-treated cells promotes the integration of foreign DNA at sites of stalled replication forks
V.5 SAHA inhibits cellular DNA repair in cisplatin-treated cells, promoting extrachromosomal DNA integration at sites of stalled DNA replication forks
CONCLUSION AND PERSPECTIVES Error! Bookmark not defined.
I. HDACi and cancer Error! Bookmark not defined.
II. The potential of HDACi in genome editing Error! Bookmark not defined.
Smith MA, Houghton P. A proposal regarding reporting of in vitro testing results. Clin Cancer Res. 2013 Jun 1;19(11):2828-33;
REFERENCES Error! Bookmark not defined.

DEDICATION

To my parents, Tatiana and Dimiter