Epigenetic editing

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APPENDICES

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Nederlandse Samenvatting

Epigenetische modificaties zoals histon-modificaties en DNA-methylering zijn gecorreleerd met genexpressie. Naast genetische mutaties zijn afwijkende epigenetische modificaties (zogenaamde epigenetische mutaties) vaak geassocieerd met aandoeningen, zoals kanker. Aangezien epigenetische factoren omkeerbaar zijn, bieden ze aantrekkelijke doelstellingen voor nieuwe therapeutische benaderingen. Bovendien zijn deze epigenetische markeringen na omzetting mogelijk stabiel en kunnen ze worden doorgegeven aan dochtercellen. Er zijn veel initiatieven gelanceerd om epigenetische enzymen te remmen, maar deze aanpak kent verschillende beperkingen, met inbegrip van hun genoom-breed effect. De bezorgdheid om de modulatie van expressie van onbedoelde genen, heeft ons ertoe gebracht gen-specifieke targeting te ontwikkelen om genexpressie te moduleren. Het doel van dit proefschrift was om epigenetische modificaties op doelgenen te herschrijven met behulp van zogenaamde Epigenetische Editing en zo hun expressie permanent te moduleren.

Na een algemene inleiding in hoofdstuk 1, behandelt het eerste deel van het proefschrift de algemene instrumenten om Epigenetische Editing te gebruiken. In hoofdstuk 2 hebben we vervolgens de meest recente vooruitgang beschreven die geboekt is bij het targeten van epigenetische effectordomeinen naar verschillende regio's in het genoom, om de genexpressie te veranderen. Inderdaad, verschillende onderzoekers hebben kunnen aantonen dat gen-gerichte Epigenetische Editing een krachtig instrument is om verschillende vragen op het gebied van epigenetica aan te pakken. Er zijn verschillende targeting domeinen ontwikkeld om epigenetische enzymen naar verschillende gebieden in het genoom te leiden. De eerste domeinen die werden gebruikt waren zink vinger eiwitten, afgeleid van zoogdiertranscriptiefactoren. Vervolgens vergrootte de ontdekking van Transcriptie Activator-achtige Effectors (TALEs) de mogelijkheden van targeting. Ten slotte, met de introductie van het innovatieve CRISPR-Cas-systeem, is het veld gegroeid tot een van de meest veelbelovende in het afgelopen decennium. In hoofdstuk 3 beschrijven we een laboratorium-protocol om met behulp van een TET2-enzyme gefuseerd aan een zink vinger gen-gericht DNA-demethylering te induceren. Tenslotte in hoofdstuk 4 hebben we de beperkingen in gerichte activatie van epigenetisch geinactiveerde endogene genen met verschillende platforms beoordeeld door de complexiteit van het epigenoom in acht te nemen. Inderdaad lijkt de chromatin micro-omgeving een van de beperkingen in targeting, met de nucleosomen als de hoofdspeler.

Het tweede deel van dit proefschrift toont de kracht van Epigenetische Editing om genexpressie bij ziekten te moduleren. In hoofdstuk 5 willen wij de dubbele rol van het RASSF1 gen in kanker onderzoeken. Het gen heeft namelijk twee promoters die twee verschillende transcripten (A en C) produceren. De eerste promoter produceert RASSF1A transcripten die een tumoronderdrukkende activiteit vertonen. De tweede promotor produceert een kleinere transcript (RASSF1C), die lijkt te zijn betrokken bij ongedifferentieerdheid. Door een van beide promoters aan- of uit te zetten kunnen we de genregulatie en zijn functies onderzoeken. In hoofdstuk 6 beschrijven we de identificatie van een nieuw gen dat overactief is in verschillende soorten kankers. Met behulp van Epigenetische Editing kunnen we aantonen dat dit gen oncogene eigenschappen heeft, en dat dit een nieuw therapeutisch doelwit biedt.

Ten slotte behandelt het laatste deel van dit proefschrift de mogelijkheid van Epigenetische Editing om duurzame genexpressie-modulatie te bereiken. In hoofdstuk 7 gebruiken we deze techniek om langduringe remming van een kandidaatgen dat een rol speelt bij chronische obstructieve longaandoeningen (COPD) te bereiken. We hebben hiertoe de expressie-remmer Super Krab Domain (SKD) en verschillende epigenetische enzymen getest. We hebben aangetoond dat een H3K9 methyleringsenzyme zijn effect van repressie kan doorgeven over celdelingen. Hoofdstuk 8 toont de mogelijkheid van het schrijven van H3K4me3 op een inactief gen om genexpressie te induceren. Dit effect is echter afhankelijk van de chromatine micro-omgeving. We hebben enkele voorwaarden beschreven die moeten worden bereikt om stabiele genexpressie van gehypermethyleerde genen te bewerkstelligen.

In hoofdstuk 9 wordt een algemene discussie gegeven over het onderzoek in dit proefschrift en we beschrijven ook enkele belangrijke factoren die de effectiviteit van de technologie beïnvloeden.

List of Publications

• J. Song, D. Cano-Rodriguez, M. Winkle, et al., “Targeted epigenetic editing of SPDEF reduces mucus production in lung epithelial cells”, AJP Lung Cellular and Molecular Physiology, 2016.

• IT. Alves, D. Cano Rodriguez, R. Bottcher, et al., “A mononucleotide repeat in PRRT2 is an important, frequent target of mismatch repair deficiency in cancer”, Oncotarget, 2016.

• D. Cano-Rodriguez & M.G. Rots, “Epigenetic editing: on the verge of reprogramming gene expression at will”, Current Genetic Medicine Reports, 2016.

• D. Cano-Rodriguez, R. A. F. Gjaltema, L. J. Jilderda, et al., “Writing of H3K4Me3 overcome s epigenetic silencing in a sustained but context-dependent manner”, Nature Communications, 2016. doi:10.1038/ncomms12284

• D. Cano, C. F. Gomez, N. Ospina, et al., “Mitochondrial DNA Haplogroups and Susceptibility to Prostate Cancer in a Colombian Population,” ISRN Oncology, vol. 2014, Article ID 530675, 11 pages, 2014. doi:10.1155/2014/530675.

• D. Cano-Rodriguez, S. Campagnoli, A. Grandi, et al., “TCTN2: a novel tumor marker with oncogenic properties”, under review in Oncotarget, 2017

• JC. Rendon, D. Cano-Rodriguez, MG. Rots, “Re-expressing epigenetically silenced genes by inducing DNA demethylation through targeting of Ten-Eleven Translocation 2 to any given genomic locus”, under review as a chapter in Methods in Molecular Biology, Springer Protocols

Biography

David Cano Rodriguez was born in Cali, Colombia (the capital of salsa dancing, which he is really good at). He obtained his high school degree at the Colegio Berchmans (a religious school, although he is not quite a believer), and graduated with honors, because he was a nerd. He then decided to pursue his career in science, and for this reason he enrolled into one of the best universities, if not THE BEST, in Colombia. In 2005, he started his bachelor in Biology at the Universidad de los Andes, where he also met the love of his life, which then became his husband. He performed his bachelor thesis project under the supervision of professor Maria Mercedes Torres, about the susceptibility to prostate cancer in the Colombian population, analyzing mitochondrial DNA ancestry. In 2010, after finalizing his bachelor studies, he moved to Europe to pursue one of his biggest dreams with his husband. He did his Master of Science in Molecular Medicine at the Erasmus Medical Center in Rotterdam, the Netherlands. His first master research project was done under the supervision of professor Guido Jenster at the department of urology, studying microsatellite instability and mutations in prostate cancer. At the end of his master he did his thesis at the Genetics department under the supervision of Professor Wim Vermeulen and Dr. Hannes Lans, analyzing the mechanisms of chromatin remodeling in DNA repair after UV damage. In 2012, after finishing his master, he got the great opportunity to obtain a PhD position at the University Medical Center Groningen, from the Rijksuniversiteit Groningen, the Netherlands, under the supervision of professor Marianne Rots. He was able to achieve sustained gene expression modulation, using the prestigious and advanced technique of epigenetic editing. In order to change the abnormal patterns of gene expression in diseases epigenetic editing was a great tool for him to achieve this goal. The results of this research are presented in this thesis. After realizing that the Netherlands was a nice country, but that the weather in Europe sucks terribly, and finally realizing that he missed his home country, he went back to Colombia with his husband on September 2016, and since December 2016 he is working at the Swiss Pharmaceutical Company Novartis, in where he is a Medical Science Liaison at the division of Oncology, in charge of the scientific and medical education of health care professionals about the new molecules being researched.

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Yours truly, David