Curriculum Vitae

Jeroen Vierstraete
Jeroen.Vierstraete@UGent.be
+32-495-11-16-54

2014-2020
Doctoral Candidate
Ghent University

2012-2014
Master of Science in Biomedical Sciences
Ghent University

2009-2012
Bachelor of Science in Biomedical Sciences
Ghent University

Examination Committee

Prof. dr. Fransiska Malfait (chair)
Ghent University, Ghent, Belgium

Prof. dr. Marina Mione
University of Trento, Trento, Italy

Prof. dr. Haico van Attikum
Leiden University Medical Center, Leiden, the Netherlands

Prof. dr. Kris Vleminckx
Ghent University, Ghent, Belgium

dr. Patrick Sips
Ghent University, Ghent, Belgium

dr. Suzanne Vanhauwaert
Ghent University, Ghent, Belgium

dr. ir. Mattias Van Heetvelde
Ghent University, Ghent, Belgium

Thesis submitted to obtain the degree 'Doctor in Health Sciences'

Faculty of Medicine and Health Sciences
Department of Biomolecular Medicine

Jeroen Vierstraete

17th December 2020

Zebrafish as a model to study DNA repair and developmental defects following loss of Brca2 or Atm

Promotor

Prof. dr. ir. Kathleen Claes
Ghent University Hospital, Ghent, Belgium

Co-promotor

Prof. dr. Anne Vral
Ghent University, Ghent, Belgium

GHENT UNIVERSITY

FACULTY OF MEDICINE AND HEALTH SCIENCES

Background and research objectives

In families with several relatives affected with breast and/or ovarian cancer, a germline pathogenic variant in a tumour suppressor gene may be present, predisposing to breast/ovarian cancer. Genetic testing is offered to uncover the presence of such pathogenic variants. The identification of a pathogenic variant may not only impact therapy choice, but also indicate that the patient is at increased risk to develop additional tumours. Therefore, individuals heterozygous for a pathogenic germline variant benefit from more intensive screenings. The pathogenic variant may be transmitted to the next generation. Therefore, some patients opt for preimplantation genetic testing to avoid transmitting this variant to their offspring.

Besides clear pathogenic variants, rare Variants of Unknown clinical Significance (VUS) are also often detected. Assays to evaluate the functionality of such variants are highly warranted. Currently available assays are based on cell work (*in vitro)*. There is a high interest in developing *in vivo* assays, as these might offer a more complete picture regarding the functionality of these VUS.

The aim of this thesis was to start optimizing such *in vivo* assays for the genes *BRCA2* and *ATM*, two breast cancer predisposition genes. For this, we used the zebrafish model organism. Zebrafish has many advantages such as a low maintenance cost, high stocking density, high fecundity, initial optical transparency and ease of genetic manipulation. In addition, many of the DNA repair pathways are largely conserved in zebrafish. In this thesis we evaluated functional read-outs and phenotypes associated with defective Brca2 and Atm in zebrafish.

Results

In **paper I**, we showed that zebrafish Brca2 is involved in the recruitment of Rad51 to the place of DNA double strand breaks. Disabling Brca2 led to complete loss of Rad51 foci formation, providing evidence that the function of human BRCA2 is conserved in zebrafish. These results show that the Rad51 foci assay could be used as an *in vivo* functional read-out to distinguish functional from non-functional Brca2 in zebrafish.

In **paper II**, we explored the possibilities of using zebrafish for *in vivo* screening of PARP inhibitor efficacies. We first showed that *brca2* deficient zebrafish are sensitive to PARP inhibitors. In addition, by using the Rad51 foci assay developed in paper I, we compared the efficacy of several PARP inhibitors. Together, these data indicate that zebrafish could speed up *in vivo* testing of novel PARP inhibitors. Lastly, we also demonstrated that zebrafish are a promising tool to model *in vivo* combination therapies that combine PARP inhibitors with irradiation.

In **paper III**, we investigated if zebrafish *atm* conserved its function as a tumour suppressor gene. For this we generated *atm* deficient fish using CRISPR-Cas9 and provided extensive characterization. In humans, *ATM* deficiency leads to Ataxia Telangiectasia, an autosomal recessive disorder characterized by ataxia, telangiectasias, neurodegeneration, premature aging, reproductive sterility, hypersensitivity to ionizing irradiation, severe immunodeficiency and a high risk of cancer. *atm* deficient zebrafish developed exclusively as infertile males. This is likely due to death of primordial germ cells, tilting the zebrafish sex determination to a male fate. Lack of mature germ cells also caused these fish to become infertile. In addition, in a *tp53* mutated background, these fish developed tumours at a much faster rate than its *atm* intact siblings. This provides evidence that the tumour suppression function of Atm is conserved in zebrafish and that *in vivo* functional read-outs can be developed.

In **review I**, we provided an overview of currently available tools to monitor DNA damage and repair in zebrafish. This review proves that zebrafish is a versatile model organism that allows *in vivo* studying of the different DNA repair pathways.

Future perspectives

In papers I and II, we developed assays to assess the functionality of Brca2 in zebrafish. As a next step, human *BRCA2* variants will be inserted into the zebrafish genome, to allow *in vivo* functional testing. We aim to engineer variants in the zebrafish genome by optimizing the novel CRISPR Prime Editing technique.

Similarly, we wish to perform functional testing of *ATM* variants. Although the “all-male” phenotype is indicative for deficient Atm (paper III), additional functional read-outs for this gene need to be developed.

In paper II we demonstrated the added value of zebrafish for screening of PARP inhibitor efficacies. Recently, polymerase θ (POLQ) inhibitors are proposed as promising novel therapies to selectively target tumour tissue while providing minimal toxicity to healthy tissues. Similar to PARP, POLQ inhibition is synthetically lethal with various DNA repair genes, including *BRCA2*, making it essential in homologous recombination-deficient cancers. Therefore, we think that zebrafish could be an attractive *in vivo* model to screen the efficacy of POLQ inhibitors.

Publications

Vierstraete J, Willaert A, Vermassen P, Coucke PJ, Vral A, Claes KBM. Accurate quantification of homologous recombination in zebrafish: Brca2 deficiency as a paradigm. *Scientific Reports. 2017 28;7(1):16518.*

Vierstraete J, Willaert A, Fieuws C, Vral A, Claes KBM. Zebrafish as an in vivo screening tool to establish PARP inhibitor efficacy. *DNA Repair (accepted).*

Vierstraete J, Willaert A, Coucke PJ, Vral A, Claes, KBM. Atm deficient zebrafish model reveals conservation of the tumour suppressor function. *Article in preparation.*

Cayuela M, Claes KBM, Ferreira M, Henriques C, van Eeden F, Varga M, **Vierstraete J**, Mione C. The Zebrafish as an Emerging Model to Study DNA Damage in Aging, Cancer and Other Diseases. *Frontiers in Cell and Developmental Biology. 2019 10;6:178.*