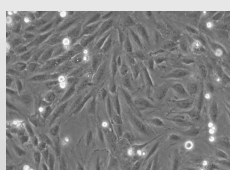


Recent studies have highlighted miR-1 as inducer of apoptosis in cardiomyocytes exposed to doxorubicin, a widely used anticancer drug. This makes miR-1 a potential target for a co-treatment to reduce the cardiotoxicity of this drug, responsible for numerous treatment stops (1,2). Apoptosis is an irreversible phenomenon occurring after the onset of significant damage in the mitochondria of cardiomyocytes. Doxorubicin is known to cause, before apoptosis, a disruption of mitochondrial energy pathways also leading to necrosis and functional alterations (3). The objective of the project is to study *in vitro* the possible involvement of miR-1 in this metabolic disturbance in order to evaluate its therapeutic potential.

## 1. Development of an *in vitro* model for cardiotoxicity assessments and microRNA transfection procedures

The first step of the project is the development of an *in vitro* model for cardiotoxicity assessments. H9C2(2-1) rat cardiomyoblasts cell line was chosen. Indeed, previous studies using this cell line showed interesting cell characteristics for drug-induced oxidative stress, apoptosis and mitochondrial disruption investigations (1). Moreover, H9C2 cell line is more suitable for transfection procedures than primary cardiomyocytes for microRNA assays (2).

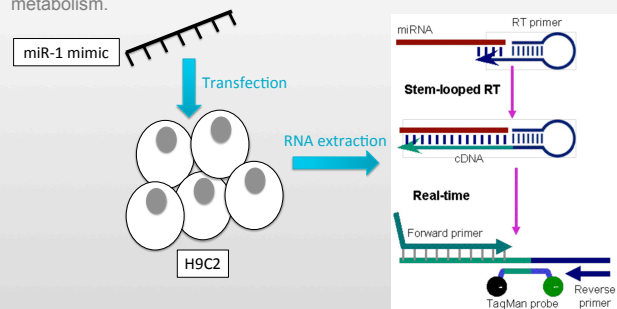
The setting of the H9C2 cell culture in the laboratory includes optimal culture conditions determination, cell line characteristics stability evaluation and validation of microRNA extraction, miR-1 expression measurement and transfection procedures.



H9C2 cell culture

## 3. Overexpression of miR-1 by transfection

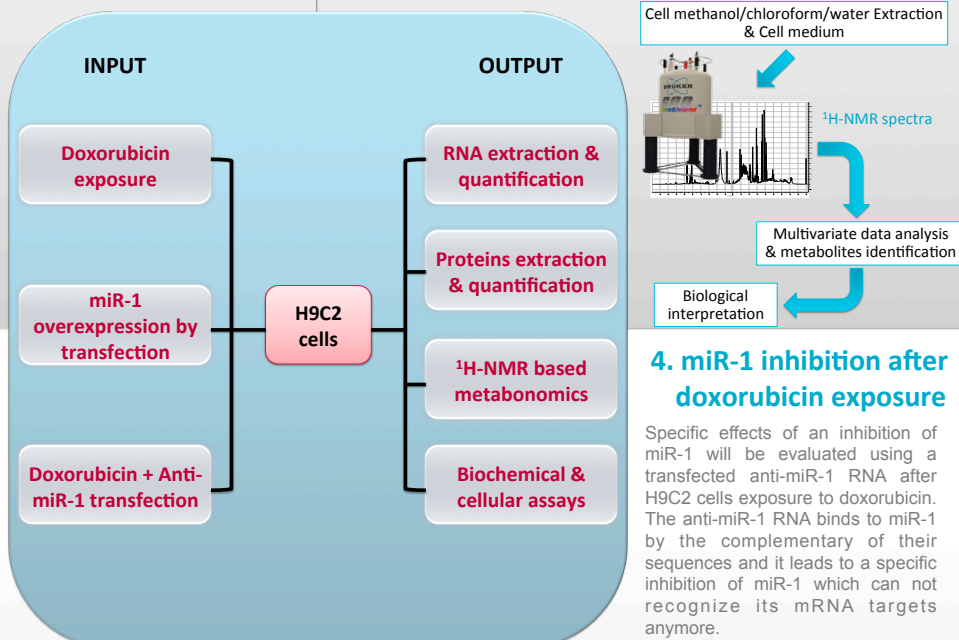
Specific effects of miR-1 on the mitochondrial metabolism will be assessed by H9C2 cells transfection with a miR-1 mimic. The miR-1 effects will be evaluated by the same approaches cited in section 2 and results will be compared with those obtained in doxorubicin-exposed cells to highlight possible miR-1 targets related to mitochondrial metabolism.



## 2. Characterization of doxorubicin-induced mitochondrial metabolism disturbances

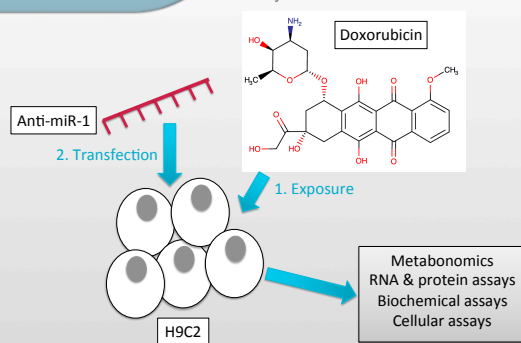
The mitochondrial perturbations induced by H9C2 cells exposure to doxorubicin will be assessed by <sup>1</sup>H-NMR based metabolomics (technical support : Dept of General, Organic and Biomedical Chemistry, Prof. Laurent, UMONS). Identified altered biochemical pathways will guide specific mRNA quantification by RT-qPCR (Institute of Pathology and Genetics, Gosselies) and proteins quantification by western blot (Dept of Molecular Biology, Prof. Declèves, UMONS). These approaches will provide a mechanistic comprehension to understand doxorubicin effects on the mitochondrial metabolism.

MiR-1 will be extracted by a specific small RNA extraction and purification technique and quantified by a RT-qPCR approach especially designed for microRNA. Several biochemical assays including oxidative stress, cellular redox state, energetic state and cellular assays will also be performed.



## 4. miR-1 inhibition after doxorubicin exposure

Specific effects of an inhibition of miR-1 will be evaluated using a transfected anti-miR-1 RNA after H9C2 cells exposure to doxorubicin. The anti-miR-1 RNA binds to miR-1 by the complementary of their sequences and it leads to a specific inhibition of miR-1 which can not recognize its mRNA targets anymore.



## References

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- Tang Y, Zheng J, Sun Y, et al. MicroRNA-1 regulates cardiomyocyte apoptosis by targeting Bcl-2. *Int Heart J*. 2009 May;50(3):377-87.
- Damiani RM, Moura DJ, Viau CM, et al. Pathways of cardiac toxicity: comparison between chemotherapeutic drugs doxorubicin and mitoxantrone. *Archives of Toxicology*. 2016 Jun 25.

