# Metabolomics analysis of time and oxygen effect on Fibrosarcoma cell line (HT1080) – model studies


## Abstract

Oxygen molecular effect on cancer cells metabolism has been a concern for decades, at the beginning with Louis Pasteur's and his understanding of glucose fermentation. In the 1930s, Otto Warburg came with his new understanding of cancer biology by introducing the alteration of cancer cells' metabolism in the presence or absence of oxygen molecules by increasing glucose uptake and producing lactate through aerobic glycolysis (Warburg Effect). For more than 100 years scientists published different studies trying to fully understand of Warburg effect and hypoxia-metabolic reprogramming effect on cancer cell progression. However, still is a lot of questions unanswered so far. For instance, why do cells choose the less sufficient way to produce ATP and lose carbon in form of lactate which is required for the biosynthetic process. What are the exact proportions of activity of cancer cells between glycolytic and the TCA cycle? And the most curious question, what is the role of stromal components in the microenvironment ? Is there any most advanced technology to answer all these questions?. By using a metabolomics platform including mass spectrometry (MS), nuclear magnetic resonance (NMR) spectroscopy, and chemometric methods to analyse a large number of metabolites present in cancer cell line samples the brighter light can be shed on identifying pathological biochemical pathways.

**Chapter 1**

Starts by introducing the state of the art in cancer biology, cancer metabolism, and the progression process and explores "Warburg effect" last consideration on metabolism reprogramming and cancer progression. Moreover, highlights the role of some essential and nonessential amino acids in cancer cell metabolism during different stress exposure to understand the metabolism alterations. In addition, the importance of oxygen molecules to cancer cell metabolism and its concentrations *in vitro* and *in vivo* terminology were described. A brief introduction also includes the contributions of NMR-based *in vitro* metabolomics and chemometric analysis as powerful and advantageous technology to investigate cultured cell line metabolome.

**Chapter 2**

In this chapter, focused on the NMR-based *in vitro* metabolomics studies on HT1080 cell line at selected oxygen concentrations as hypoxia 1%, normoxia 6%, and hyperoxia 21% by using 1D $^{1}$H NMR spectroscopy based on cells extracts (intracellular) and post cultured medium (extracellular), including sampling during incubation in the time intervals. Latest studies suggested that across many types of tumors after assessment of their degree of hypoxia using a set of genetic hypoxia markers revealed a great variation among tumours type in terms of potential hypoxia influence. Moreover, the normoxia term very often used 21% of oxygen concentration in *in vitro* studies, which is much higher than physiological oxygen concentration. Therefore, there is a need to develop a novel methodology to mimic the oxygen pressure on body tissues, that could distinguish the differences between oxygen environment saturation (hypoxia ≤ 1 and normoxia 6% and hyperoxia 21%). Hence, the metabolic profile information was collected by using 1D $^{1}$H NMR spectra from each interval of incubation time (12h, 24, and 36h with respect to the control samples) of fibrosarcoma cell line including intra and extracellular metabolites analysis. The chemometric analyses were applied to determine the potential of metabolites for discrimination purposes between interval incubation time. Besides, increasing the oxygen content and the possibilities of metabolite changes could be performed, namely at hypoxia at 1%, normoxia at 6%, and hyperoxia at 21% condition.

**Chapter 3**

In this chapter of the doctoral dissertation, uses novel terms and methodology for inducing hypoxic-reoxygenation and normoxic-deoxygenation *in vitro* models to screen HT1080 cell line by looking at extracellular and intracellular metabolome. Behind this concept, tumor hypoxia arises from the rapid and uncontrolled proliferation of cancer cells, leading to increased acquisition of nutrients and oxygen to meet the energy demands. However, a quick depletion of oxygen and nutrient supply developed in parts of the tumour. Means, there is a transition of cells from sufficient oxygen and nutrients supply to hypoxic conditions with insufficient oxygen and nutrients supply normoxic-deoxygenation. In another hand, the hypoxic region within tumours generates different strategies to acquire an adequate quantity of oxygen and nutrients for cancer progression. For instance, induce the production of angiogenic proteins to build new vessels for blood flow and cancer cell mobility through

epithelial-mesenchymal transition (EMT) and metastasis. Thereby, cancer cells become normoxic cells with a sufficient supply of oxygen and nutrients (hypoxic-reoxygenation). Both these terms develop different metabolic phenotypes of cancer cells, so the combination of the analyses for intracellular and extracellular metabolites including normoxic vs. deoxygenized normoxic cells (DNCs) and hypoxic vs. reoxygenized hypoxic cells (RHCs) through incubation time experiments were conducted.