Abstract (English)

Niclosamide, a FDA- approved drug against tapeworm, has been discovered to have anti-proliferative activity in several cancer types [1-6]. Due to its low bioavailability of only 10% [7] a new strategy to reformulate and encapsulate the drug has been performed in the SPSE group to improve its delivery efficacy [8]. Nanoparticles with a new pro-drug: the niclosamide stearate nanoparticles (NSNPs) has been designed and formulated. The NPs were targeted to the folate receptor (FOLR), based on the fact that studies showed elevated levels of FOLR in malignant cancer cells [9]. The NP design was further inspired by the LDLs, which are highly internalized in the TNBCs [10-12]. Thus, the overall goal of this phD project, was to examine the utility of the NPs and to target the most aggressive breast cancer cells (BCCs) and BCSCs, an approach we call "putting the drug in the cancer's food".

Chapter 1 is an overall introduction to the thesis that provides the background and the motivation for this thesis including the overall goal, specific aims and associated hypotheses of the Ph.D. project.

Chapter 2 contains an evaluation of the LDLR and FOLR expression levels and the uptake level of DiI-LDLs, DiI-NPs with triolein (stealth (SNPs) and FOLR targeted NPs (FNPs)) in a panel of BCCs and BCSCs. In addition, the role of the cholesterol pathway in the BCSC development was assessed. The results overall demonstrated promising potential in delivering our NSNPs to BCCs and BCSCs. Elevated LDLR expression and LDL uptake were predominant in the TNBC cells and exhibited selectivity towards the BCSCs. Both LDLs and SNPs were able to enter the spheres despite the 3D barrier, while LDLs even had a better internalization of the NPs. LDL also showed to be essential for the stem cell formation while statins had the opposite effect. This highlighted the potential of using the LDLR as a delivery route to target these BCSCs. In a similar screen, FOLR expression was predominant in luminal BCCs and exhibited selectivity towards BCSCs. Furthermore FOLR targeting improved the uptake of the NPs in the luminal BCCs and CSC-like epithelial and mesenchymal tumorigenic TNBC cells.

Chapter 3 includes an examination of the NSNPs anticancer effect (stealth (SNSNPs) and FOLR targeted (FNSNPs) in BCC and BCSCs compared to niclosamide. The primary mechanism

of the NSNPs and niclosamide *in vitro* was to reduce the metabolic activity. Thus, an effective response to the NSNPs was achieved, as both promoted a cytostatic effect similar to niclosamide, while reducing the off-target efficacy. Targeting to the FOLR showed even in some cases to increase the efficacy in the BCCs. However, the NSNPs only induced a maximum of 75- 80 % cell death, and so failed to completely eliminate the BCCs. Both NSNPs reduced the mammosphere formation efficacy and metabolic activity similar to niclosamide in BCSCs, which were treated just after seeding. A less effect was yet obtained when 3D mammospheres were formed, which made the BCSCs more resistant to the NSNPs. Nevertheless, targeting to the FOLR also here revealed to reduce the off-target toxicity.

Chapter 4 contains an examination of the cellular entry pathway of the NPs and the specific intracellular mechanism involved in the cytotoxic effect of the NSNPs in BCCs. SNPs and FNPs were internalized in almost 100% cells within 24h. Due to the competitive effect by free folic acid (FA) in the media, it was shown that the uptake of both NPS was hindered by occupation of the FOLR in the BCCs. The intracellular pathway of the SNPs involved a combination of the clathrin-dependent and clathrin-independent pathways, while FNPs were internalized by the clathrin-dependent pathway alone. When inside the BCCs, both NPs co-localized with the lysosome. By examining the intracellular mechanism, the results showed that both NSNPs induced the same amount of cell growth arrest and apoptosis as the niclosamide drug in BCCs, while the NSNPs reduced[13] off-target efficacy. More specifically, the apoptosis induction was induced by a caspase-dependent and caspase-independent way, while an induction of autophagy and lysosome membrane permeabilization (LMP) was also visible.

Chapter 5 includes the overall summary, conclusion and future scope.