Targeting lipid metabolism in cancer – through translationally edged clinical trials

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Introduction

Breast cancer is the most common form of cancer worldwide, with an incidence of over one million new diagnoses yearly [1, 2]. In recent time, the mortality has decreased but breast cancer is still one of the most common death causes among young women and there is a great need of the development of new medicines and treatment regimens [2]. There is increasing evidence that tumor cell lipid metabolism plays a crucial role for cancer development, and targeting the lipid metabolism is believed to have a central role in development of new cancer drugs and biomarkers.

An important signaling pathway in lipid metabolism is the mevalonate pathway, leading to the formation of cholesterol, steroid hormones and isoprenoids. The rate-limiting enzyme of the mevalonate pathway, hydroxy-methyl-glutaryl coenzyme A reductase (HMGCR), is the target of statins, a group of drugs widely used for prevention of cardiovascular diseases. Statins competitively inhibit HMGCR, leading to an inhibition of the de-novo synthesis of cholesterol, and consequently a lowering of the plasma levels of cholesterol [3]. In vitro studies have shown that statins exert anti-tumoral effects by reducing tumor cell proliferation and increasing apoptosis [4]. In vivo breast cancer studies have shown a statin-induced reduced tumor cell proliferation and a reduced risk of breast cancer recurrence [5-7].

The overall aims of this thesis are:

- To obtain an overall understanding of the importance of the mevalonate pathway for the lipid metabolism in cancer cells
- To identify anti-tumoral effects of a statin-induced inhibition of the mevalonate pathway
- To investigate statins as an additional cancer treatment in a clinical phase II trial

Materials and methods

Project I-II is based on a window-of-opportunity clinical phase 2 trial, the MAST (MAmmary cancer and STatins)-study. A total of 50 patients were included and prescribed a high dose of atorvastatin (80mg/day) for two weeks during the treatment free window between diagnosis and surgery. Before start of statin treatment a core needle biopsy was taken from the tumor and blood samples were collected. After two weeks of treatment, tumor tissue was retrieved at the standard surgical procedure, and at the same time, new blood samples were collected. A statin-induced change of proliferation served as a primary endpoint [6]. Project I of this thesis aims to understand the mediation of the statin-induced anti-proliferative effect better. The protein expression of the cell-cycle regulators cyclin D1 and p27 was evaluated by immunohistochemistry on paired samples of formalin fixed paraffin embedded tumor tissue, sampled before and after atorvastatin treatment. In project II, frozen tumor samples pre- and post-atorvastatin treatment have been analyzed by extracting lipids from the tumor samples. Cholesterol levels were then measured using a cholesterol quantification assay in order to evaluate changes in the cholesterol levels. In vitro experiments on MCF7-cells treated with
atorvastatin were performed for comparison on the cellular level. The expression of the LDL-receptor was analyzed by immunohistochemistry on formalin fixed paraffin embedded tumor tissue, pre- and post-atorvastatin treatment.

Project III is based on a population-based prospective cohort study, the Malmö Diet and Cancer Study (MDCS). Tumor expression of HMGCR was assessed by immunohistochemistry on tissue microarrays from 910 women diagnosed with primary breast cancer between the years of 1991-2010. Breast-cancer specific mortality according to HMGCR expression and use of cholesterol lowering medications was analyzed.

Project IV will be a descriptive publication of a clinical phase II trial – ABC-SE – where the effect and tolerability of atorvastatin in combination with endocrine treatment among patients with advanced breast cancer will be compared to standard endocrine treatment. The goal of this study is to understand the mechanisms behind resistance to endocrine treatment of breast cancer, and also to test the hypothesis that the addition of statins will enhance the effect of the endocrine treatment.

Results from completed studies I-III

Project I revealed a significant down-regulated expression of the oncogene cyclin D1 and a significant up-regulated expression of the suppressor p27 following two weeks of statin treatment [8].

Project II showed a statin-induced upregulation of the LDL-R and preserved intra-tumoral cholesterol levels. In agreement, the in vitro experiments showed no significant changes of the intracellular cholesterol levels after atorvastatin treatment, but a higher expression of the LDL-R, however non-significant.

In project III, HMGCR expression was associated with prognostically adverse tumor characteristics although no significant associations were observed for breast cancer related mortality. Among breast cancer patients on cholesterol lowering medications, no or weak HMGCR expression appeared clinically favorable.

Conclusions

Project I and II have shown tumor effects after two weeks treatment with atorvastatin and contribute to the elucidation of how statins impact on breast cancer. Larger clinical trials are needed to address the potential role of statins as anti-cancer drugs. In project III, a high HMGCR expression was associated with more aggressive tumor characteristics. Further studies are needed to establish the role of HMGCR as a predictive biomarker.