Report NCU grant

Report submission date: 24th of May 2013

Main applicant: Anne-Lise Børresen-Dale

Project title: HER2 positive cancers and drug response: a Finnish-Norwegian collaboration

NCU grant received (€): 90.000

Project commencement and completion dates: 31st Dec 2013

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1. Brief description of the project, written in a language understandable to non-scientists (Maximum length: 100 words)

20% of all BCs have an amplification of the chromosome region 17q12-q21, leading to overexpression of the gene product of the HER2/ERBB2 gene. Such patients are known to have aggressive BCs, and are currently treated with the humanized monoclonal antibody Trastuzumab (Herceptin). However, more than half of the Her2+ BCs respond poorly or become resistant to the drug. Studying the molecular mechanisms of Her2 positive BCs to learn more about the disrupted pathways in these cancers at mRNA, miRNA, DNA, protein and functional level is therefore highly desirable. In this proposed project we seek to increase the knowledge of the molecular mechanisms and the signalling pathways involved in Her2+ patients by integration of molecular profiling data patients with functional data from in vitro models.

2. Summarize the major findings of the project (Maximum length: 400 words)

The HER2 amplicon at 17q12 is large and contains multiple genes, and we have systematically explored the role of the *HER2* co-amplified genes in breast cancer development and Trastuzumab resistance. We integrated aCGH data of the HER2 amplicon from 71 HER2 positive breast tumors and 10 cell lines with systematic functional RNA interference analysis of 23 core amplicon genes with several phenotypic endpoints in a panel of Trastuzumab responding and non-responding HER2 positive breast cancer cells. Silencing of *HER2* caused a greater growth arrest and apoptosis in the responding compared to the non-responding cell lines, indicating that the resistant cells are inherently less dependent on the HER2 pathway. Several other genes in the amplicon also showed a more pronounced effect when silenced; indicating that expression of *HER2* co-amplified genes may be needed to sustain the growth of breast cancer cells. Importantly, co-silencing of *STARD3, GRB7, PSMD3* and *PERLD1* together with *HER2* led to an additive inhibition of cell viability as well as induced apoptosis. These studies indicate that breast cancer cells may become addicted to the amplification of several genes that reside in the HER2 amplicon. The simultaneous targeting of these genes may increase the efficacy of the anti-HER2 therapies and possibly also counteract Trastuzumab resistance.

In order to characterise the role of miRNAs in the regulation of HER2-signaling in breast cancer, we have performed miRNA gain-of-function assays by screening two HER2-positive cell lines (KPL-4 and JIMT-1) with Dharmacon miRNA mimic library consisting of 810 human miRNAs. Cell viability was measured with CellTiter-Glo® (Promega) whereas HER2, phospho-AKT, phospho-ERK1/2, cell proliferation (Ki67) and apoptosis (cPARP) were detected with specific antibodies using protein lysate microarrays. The screens revealed several miRNAs, which downregulated HER2, inhibited growth and affected the activation of AKT and ERK1/2. We identified 25 novel HER2 regulating miRNAs, of which nine bound directly to the 3'UTR region of HER2. In addition, we identified miRNAs which were associated with better survival in clinical breast tumor samples (manuscript submitted).

We have also screened thirteen HER2+ breast cancer cell lines with 22 compounds targeting HER2, the EGFR family, or HER2 downstream signaling pathways. Several compounds inhibited cell growth statistically more efficiently than Trastuzumab over the whole cell panel. Several compounds were found to inhibit growth also in the Trastuzumab non-responsive cell lines. In order to understand the molecular mechanisms behind the differences in response, mRNA data together with copy number changes and *PIK3CA* mutation statuses were analyzed for all cell lines. The cells were grouped according to Trastuzumab response, PIK3CA, or PTEN status and further analyzed based on drug screening results. The mechanisms of action of these drugs will be further studied.

3. Describe how the project has increased our knowledge of the prevention, cause and/or cure for cancer (Maximum length: 150 words)

The proposed breast cancer research project on Her2+ cancers and drug response is highly relevant for the NCU strategy. The success of the project is dependent on close collaboration between Norway and Finland. The project will integrate data from multiple levels in a systems biology approach, and we believe that increased knowledge of Her2+ cancers and their drug resistance will arise in this project. Hopefully, new therapies for further in vivo testing will be presented. This work has been actively conducted in both countries, and post docs from both laboratories have visited the other country for research training.

4. Outline how Nordic cooperation has added value to this project (Maximum length 100 words)

The funding from NCU in 2012 has strengthened the collaboration between the two labs in Norway and Finland. A two days meeting with the people involved for the project took place in Turku in May 2012, and several shorter exchange visit of members of the two labs have been important for the continuation and follow up of the projects. A Finnish postdoc from VTT Medical Biotechnology was hired at the Department of Genetics in Oslo for two years (funded elsewhere) and is involved in the project.

5. Publications resulting from this grant

Kleivi Sahlberg K, Hongisto V, Edgren H, **Makela R, Hellström K, Sahlberg N, Due EU, Vollan MV,** Wolf M, **Børresen-Dale AL, Perala M,** Kallioniemi O. "*The HER-2 amplicon includes several genes required for growth and survival of Her-2 positive breast cancers".* Molecular Oncology Epub 2012 Nov 24.

One master thesis with the title "Expression and function of microRNAs in HER2+ breast cancer" was defended at the Department of Chemistry, Biotechnology and Food Science at the Norwegian University of Life Science. Suvi-Katri Leivonen and Kristine Kleivi Sahlberg were supervising the project.

In addition 2 oral presentations and 8 posters from this project has been presented at national and international conferences.