NCU – Summative report for 2013

Report submission date: 24th of March 2014

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

NCU grant received (€): 70.000

Project commencement and completion dates: 31st of Desember 2013

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1. Briefly describe the project in a language understandable to non-scientists (max. 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 (max. 400 words)

Three-dimensional cell culture models have become increasingly popular and are suggested to be better models than two-dimensional monolayers due to improved cell-to-cell contact and structures that resemble in vivo architecture. We compared the drug responses in HER2 positive cells when grown in two dimensions, in poly(2-hydroxyethyl methacrylate) induced anchorage-independent three-dimensional models, and in Matrigel three-dimensional cell culture models by screening 102 compounds. Large variations in drug responses were observed between the models indicating that comparisons of culture model-influenced drug sensitivities cannot be made based on the effects of a single drug. However, cells grown on Matrigel were significantly more sensitive to drugs than cells grown in two-dimensional cultures, while the responses of cells grown in poly(2-hydroxyethyl methacrylate) resembled those of the two-dimensional cultures (PLoS One 2013).

We have systematically explored the role of the *HER2* co-amplified genes at 17q12 in breast cancer development and trastuzumab resistance. Silencing of *HER2* caused a greater growth arrest and apoptosis in the trastuzumab 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 (Mol Oncol. 2013).

The miRNA regulation of HER2-signaling is characterized by miRNA gain-of-function assays with miRNA mimic library consisting of 810 human miRNAs. The levels of HER2, phospho-AKT, phospho-ERK1/2, cell proliferation (Ki67) and apoptosis (cPARP) were analyzed with reverse-phase protein arrays. Rank product analyses identified 38 miRNAs as inhibitors of HER2 signaling and cell growth. We also characterized miRNAs directly targeting HER2 and identified seven novel miRNAs (miR-552, miR-541, miR-193a-5p, miR-453, miR-134, miR-498, and miR-331-3p) as direct regulators of the HER2 3'UTR. These results give mechanistic insights in HER2 regulation which may open potential new strategies towards prevention and therapeutic inhibition of HER2-positive breast cancer (Mol Oncol. 2014).

We have also screened thirteen HER2+ breast cancer cell lines with 22 compounds targeting HER2 signaling pathways. The molecular mechanisms related to treatment response were sought. Several compounds inhibited cell growth statistically more efficiently than trastuzumab over the whole cell panel, interestingly, an Akt1/2 kinase inhibitor was highly efficient in cells that did not respond to trastuzumab. To search for response predictors, genomic and transcriptomic profiling, *PIK3CA* mutations and *PTEN* status were associated to the drug responses and 32 genes involved in the response of 13 compounds were identified (manuscript submitted).

In addition we have several collaborative projects between Medical Biotechnology VTT and Department of Genetics OUS has resulted in publications.

3. Describe how the project has increased our knowledge of the prevention, cause and/or cure for cancer (max. 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 (max. 100 words)

The funding from NCU in 2013 has strengthened the collaboration between the two labs in Norway and Finland. 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 has worked two years at the Department of Genetics in Oslo (funded elsewhere) and is involved in the project. A PhD student at Department of Genetics (funded elsewhere) has been involved in the project in the three years it has been ongoing, and is delivering her PhD thesis in April 2014.

5. Publications resulting from the NCU research grant

Scientific articles resulted from the Finnish-Norwegian collaboration:

1. High-throughput 3D screening reveals differences in drug sensitivities between culture models of JIMT1 breast cancer cells. Hongisto V, Jernström S, Fey V, Mpindi JP, Kleivi Sahlberg K, Kallioniemi O, Perälä M. PLoS One. 2013 Oct 23;8(10):e77232. doi: 10.1371/journal.pone.0077232
2. The HER2 amplicon includes several genes required for the growth and survival of HER2 positive breast cancer cells. Sahlberg KK, Hongisto V, Edgren H, Mäkelä R, Hellström K, Due EU, Moen Vollan HK, Sahlberg N, Wolf M, Børresen-Dale AL, Perälä M, Kallioniemi O. Mol Oncol. 2013 Jun;7(3):392-401. doi: 10.1016/j.molonc.2012.10.012. Epub 2012 Nov 24. *(Reported in 2012)*
3. Identifying microRNAs regulating B7-H3 in breast cancer: the clinical impact of microRNA-29c. Nygren MK, Tekle C, Ingebrigtsen VA, Mäkelä R, Krohn M, Aure MR, Nunes-Xavier CE, Perälä M, Tramm T, Alsner J, Overgaard J, Nesland JM, Borgen E, Børresen-Dale AL, Fodstad O, Sahlberg KK, Leivonen SK. Br J Cancer. 2014 Feb 27. doi: 10.1038/bjc.2014.113.
4. Individual and combined effects of DNA methylation and copy number alterations on miRNA expression in breast tumors. Aure MR, Leivonen SK, Fleischer T, Zhu Q, Overgaard J, Alsner J, Tramm T, Louhimo R, Alnæs GI, Perälä M, Busato F, Touleimat N, Tost J, Børresen-Dale AL, Hautaniemi S, Troyanskaya OG, Lingjærde OC, Sahlberg KK, Kristensen VN. Genome Biol. 2013 Nov 20;14(11):R126.
5. High-throughput screens identify microRNAs essential for HER2 positive breast cancer cell growth. Leivonen SK, Sahlberg KK, Mäkelä R, Due EU, Kallioniemi O, Børresen-Dale AL, Perälä M. Mol Oncol. 2014 Feb;8(1):93-104. doi: 10.1016/j.molonc.2013.10.001. Epub 2013 Oct 11.
6. Deregulation of cancer-related miRNAs is a common event in both benign and malignant human breast tumors. Tahiri A, Leivonen SK, Lüders T, Steinfeld I, Ragle Aure M, Geisler J, Mäkelä R, Nord S, Riis ML, Yakhini Z, Kleivi Sahlberg K, Børresen-Dale AL, Perälä M, Bukholm IR, Kristensen VN. Carcinogenesis. 2014 Jan;35(1):76-85. doi: 10.1093/carcin/bgt333. Epub 2013 Oct 8.

In addition 3 posters are presented at international conferences:

1. 4/2013 AACR annual meeting, Washington DC, U.S.A. High-throughput 3D screening reveals differences in drug sensitivities between culture models of JIMT1 breast cancer cells.
2. 8/2013 The 23rd annual Biocity symposium, Turku, Finland. High-throughput 3D screening reveals differences in drug sensitivities between culture models of JIMT1 breast cancer cells
3. 9/2013 European cancer congress, Amsterdam, Netherlands. High-throughput 3D screening reveals differences in drug sensitivities between culture models of JIMT1 breast cancer cells