# Meeting Report: The Second Norwegian Cancer Symposium "Frontiers in Cancer Stem Cell Research: From Basic Science Towards a Cure", Oslo, Norway.

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**Key words:** Cancer stem cells, stem cell-microenvironment, stem cell plasticity

**Abbreviations:** CSC: cancer stem cell, EMT: epithelial to mensenchymal transition, APC: adenomatous polyposis coli, BrdU: bromo-2-deoxyuridine, GFP: green fluorescent proten, HSC: hematopoietic stem cell, CML: chronic myeloid leukaemia, IFN: interferon, MM: multiple myeloma

**Abstract**

The "Second Norwegian Cancer Symposium" was held in Oslo, Norway, the 2nd to the 4th of December 2009.The meeting, which was titled "Frontiers in Cancer Stem Cell Research: From Basic Science Towards a Cure", was held in a 100 year old building in a

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winter-dressed hill overlooking Oslo, where nearly 300 researchers met to discuss the latest developments in the field.

There were many interesting topics covered, and a recurring theme during the meeting was the importance of the niche for cancer stem cells (CSCs) and their activity. A part of the meeting was also dedicated to the field of epithelial-mesenchymal transition (EMT) and its involvement in CSC development.

In this meeting report we will only be able to give some highlights from the meeting, and discuss the progress towards CSC-specific therapies.

**Characterisation of cancer stem cells**

The meeting started with Pier Giuseppe Pelicci (Milan) presenting his groups work on the regulation of self-renewing divisions in CSCs (1). As cancer model systems they used mice over-expressing the oncogene *ErbB2* and mice lacking the *p53* tumour suppressor. They show that wild type stem cells have a preference towards asymmetric division, keeping the number of stem cells constant, whilst ErbB2 over-expressing CSCs predominantly undergo symmetric divisions. The unlimited symmetric divisions of the CSCs, which are caused by inactivation of *p53*, resulted in an increased number of CSCs in tumour tissues. Furthermore, Pelicci et al. show that Nutlin3, which inhibits MDM2-dependent p53 degradation (2), restores p53 function in both stem cells and CSCs, however, only the number of CSCs is reduced upon re-expression of p53, with no effect on normal stem cells. This finding suggests that Nutlin3 reduces the frequency of self-

renewal in CSCs by switching their mode of division from symmetric to asymmetric. In addition, Nutlin3 treatment resulted in reduced tumour size *in vivo*, with no effect on cell growth or apoptosis in bulk tumour cells. Thus, Nutlin3 targets the CSCs, and can potentially be used in CSC therapy.

**The importance of the niche, - proliferating and quiescent stem cells**

As researchers are gaining further understanding about stem cell mechanisms in various tissues, a new alternative stem cell model emerges, in which sub-populations of quiescent and active stem cells co-exist (3).

Hans Clevers (Utrecht) presented their recent findings on the multi-potent proliferative Lgr5+ stem cells of the gut (4, 5). Using a sophisticated rainbow mouse knock-in system, they show that the Lgr5+ stem cells are able to self-renew, as well as to generate all the cells of the gut, thereby giving rise to fully mature crypts *in vivo*. Surprisingly, even though the stem cells are highly proliferative, they show no sign of exhaustion. Furthermore, Lgr5 + stem cells also have the ability to generate a complete self-renewing intestinal epithelial system *in vitro* ("minigut") (5). The production of these "miniguts" from single Lgr5+ stem cells is an inefficient process. However, the efficiency is highly increased by cultivation of cell doublets, where a Lgr5+ stem cell is accompanied by a Paneth cell. Interestingly, these cells are in close proximity *in vivo*, indicating that the Paneth cell functions as an important niche for the Lgr5+ stem cell.

Clevers also convincingly showed that Lgr5+ stem cells take on a CSC phenotype upon deletion of the tumour suppressor gene adenomatous polyposis coli (APC) (6). In mouse model systems, Lgr5+ stem cells lacking APC induce rapidly growing tumours in the entire gut. In contrast, progenitor cells lacking APC produce small growths, which stop expanding over time. Loss of APC in single Lgr5+ stem cells is associated with induction of intracellular β-catenin expression (marker for intestinal proliferation), and subsequent production of more stem cells. Thus, de-regulation of APC appears to result in generation of CSCs. Interestingly, the Paneth cell produces signalling molecules such as EGF, Wnt and Notch, making these molecules prime therapeutic targets for cancer of the gut.

Andreas Trumpp (Heidelberg) reported his group's findings on dormancy in the HSC system. It is becoming evident that the HSC compartment is split in two; a population of active stem cells, which maintain homeostasis, and a smaller sub-population of dormant stem cells, which can be activated upon crisis. Trumpp and colleagues have performed sophisticated long term label retention experiments in mice, where they utilise either 5-bromo-2-deoxyuridine (BrdU) labelling of the HSCs, or a histone-2B-green fluorescent protein (GFP) transgenic mouse model (7). Using these two systems, they show that the dormant HSCs are estimated to divide a few times during the life span of a mouse, while the more active counterparts divide more frequently. The dormant HSC is quiescent, as indicated by the fact that it is negative for the proliferation marker Ki67, and the replicative machinery is turned off. These dormant HSCs are activated upon injury. On the contrary, the active HSCs (which are Ki67 positive) have limited self-renewal

capacity, as demonstrated by serial transplantation studies. Although the exact mechanism has not been unravelled, Trumpp hypothesised that upon injury there is a depletion of mature blood cells, inducing a strong feedback loop that activates the dormant HSCs. Once the progenitor pool has been repopulated, the feedback loop becomes less active and is eventually switched off.

**Basic science towards a cure**

Cancer therapies have traditionally been focused on reducing the sheer size of the tumour. However, traditional therapies do not necessarily target the CSCs, which in many cases are thought to be responsible for relapse and metastasis. Generation of new cancer therapies which target the CSCs are therefore thought to improve the overall patient survival.

Chronic myeloid leukaemia (CML) is a CSC disease, caused by the Philadelphia chromosome (BCR-ABL). Whilst close to 100% of CML patients respond to treatment with imatinib, which targets the resulting fusion protein, the majority of the patients relapse once the drug is withdrawn, or once the patient develops resistance to the drug. Imatinib as a single agent targets and degrades the fusion protein BCR-ABL in dividing cells, but it is unable to target the dormant CML stem cell. Interestingly, Trumpp presented evidence that the dormant HSCs/CSCs can become activated and enter the cell cycle upon interferon (IFN)α treatment, thus sensitizing the cells to chemotherapy (8). In a CML mouse model, priming with IFNα followed by imatinib treatment resulted in

complete remission and no relapse after imatinib withdrawal. Similarly, 5 out of 6 CML patients treated with IFNα and imatinib have cleared the disease (9).

William Matsui (Baltimore) presented his data focused on CSCs in differentiated hematopoietic tumours, such as multiple myeloma (MM) and lymphomas. Many MM patients respond well to the cancer treatment, however, their overall survival fraction is no better than the survival for patients that receive no treatment. This discordance between response to treatment and clinical outcome shows the importance of developing new strategies to treat MM. Matsui and colleagues have identified two cell types in MM; plasma cells (CD138+) and a smaller percentage of B-cells (CD138 -, CD27+) (10). In contrast to the cancerous plasma cells, the small population of cancerous B-cells is clonogenic and can be serially transplanted in immune deficient mice where they also give rise to the plasma cells. Furthermore, the cancerous B-cells are resistant to irradiation, have highly active membrane pumps, and high levels of the detoxifying enzyme aldehyde dehydrogenase. Together this indicates that the B-cells have CSC characteristics, and may be responsible for tumour relapse. Targeting the cancerous B-cells may improve the outcome for MM patients.

**Epithelial-mesenchymal transition and cancer stem cells**

A part of the meeting was dedicated to the field of EMT. During the EMT program, transformed epithelial cells loose their epithelial traits, and instead gain mesenchymal traits that seem to facilitate metastasis.

The finding that EMT is implicated in the development of some CSCs, suggests that therapies blocking or reversing the EMT process may sensitize resistant CSCs to traditional cancer treatments. Transcription factors key to the EMT process are generally expressed in the embryo, and often re-expressed in cancers. In this respect, Sendurai Mani (Houston) and colleagues have interestingly shown that the expression of the transcription factor Foxc2 induces EMT in human mammary epithelial cells (11). The expression levels of Twist and Snail are also increased during the EMT process. Furthermore, cells undergoing EMT generate cells that display CSC characteristics, such as increased ability to form spheroids, to self-renew and to form tumours in mice (12).

Interestingly, expression of Foxc2 is correlated with highly aggressive basal-like breast cancers, indicating a key role for Foxc2 in cancer invasion and metastasis. Thus, Foxc2 might prove to be a highly specific marker for this cancer subtype in the future.

Thomas Brabletz (Freiburg) focused his talk on the transcriptional repressor ZEB1, which is also an inducer of EMT. He convincingly showed that knock down of ZEB1 reverses the EMT process, and induces epithelial characteristics in tumour cells, more specifically inhibition of cell migration and reduced invasiveness and metastasis (13). Brabletz and co-workers have further elucidated the mechanisms involved in these processes, and have found that ZEB1 directly suppresses the transcription of the microRNA-200 family members miR-141 and miR-200c, which are inhibitors of EMT (14). These findings have clinical relevance, as others have shown that ZEB1 is associated with increased drug

resistance and poor prognosis in pancreatic cancer (15). miR-141 and miR-200c could therefore be key candidates for future miRNA-based cancer therapies.

A better understanding of the EMT process at the molecular level will give new insights into the mechanisms of cancer progression and may suggest new opportunities for therapeutic intervention. Snail, Twist and ZEB1, which are components active in the EMT pathway, are key candidate targets for CSC therapies.

**Summary**

Cancer stem cells are likely to arise through several different mechanisms. As the field is gaining a deeper understanding of the mechanisms involved in normal stem cell and CSC development, maintenance and regulation, it is becoming evident that there are a number of different approaches to developing CSC therapies. Whilst the CSCs themselves are currently key targets for CSC therapy, the topics discussed in this meeting demonstrate that future CSC therapies will also target the surrounding niche and the EMT program.

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