PROGRAM

The 6th International Conference
'Notch Targeting in Cancer'
Santa Marina Hotel, Mykonos, Greece
15 - 16 June 2016

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Wednesday 15th June 2015

4.00 - 4.30 pm **Registration**

4.30 - 4.35 pm **Welcome: Agamemnon Epenetos**

SESSION 1 Chairman: Adrian Harris

4.30 - 5.00 pm **Keith Brennan**

Highlights of the 5th Meeting, Notch Targeting in Cancer, June, 2015

5.00 - 5.30 pm Anushka Dongre[1,2], Carl Pierce[1] and Barbara A. Osborne[1]
[1]University of Massachusetts, Amherst, MA,[2]Whitehead Institute for Biomedical Research,Cambridge, MA

Non-Canonical Notch Signaling in CD4+ T Cells

The Notch signaling pathway plays a crucial role in regulating immune cell fate decisions and peripheral T cell function. Activation of the Notch receptor is triggered after binding of cognate ligands, Delta-like or Jagged, resulting in a series of proteolytic cleavage events that culminate in the release of the intra-cellular domain of Notch by γ-secretase. The active, intra-cellular domain of Notch ($N1^{IC}$) migrates to the nucleus and interacts with its canonical binding partner RBP-Jk, which is a repressor of transcription. Recruitment of co-activators to the $N1^{IC}$ - RBP-Jk complex, converts RBP-Jk from a repressor to an activator of transcription, leading to the expression of downstream target genes. In addition to RBP-Jk, $N1^{IC}$ has also been shown to interact with other proteins in the cytoplasm and the nucleus, suggesting that Notch may exert some of its function in a non-canonical, RBP-Jk- independent fashion. Data from our lab demonstrates Notch regulates several peripheral T cell responses in an RBP-Jk independent fashion.

Our data suggest that Notch interaction with other signaling pathways, including NF-kB and mTORC2, may regulate several key functions of Notch in peripheral $CD4^+$ T cell activation and function. The details of these non-canonical interactions and the possible consequences of non-canonical Notch signaling in diseases such as autoimmunity and cancer will be the focus of my presentation.

5.30 - 6.00 pm Rajwinder Lehal 1 5, Viktoras Frismantas 2, Viktoria Reinmüller 1, Jean-Pierre Bourquin 2, Gerardo Turcatti 3, Sylvain Loubery 4, Marcos Gonzalez-Gaitan 4, Michael Bauer 5, Dirk Weber 5 and Freddy Radtke 1 5

1 Ecole Polytechnique Fédérale de Lausanne (EPFL), School of Life Sciences, ISREC, Station 19, 1015 Lausanne, Switzerland ; 2 University Children's Hospital Zurich, Division of Pediatric Oncology, August-Forelstrasse 1, 8008 Zürich, Switzerland; 3 Biomolecular Screening Facility, EPFL, Lausanne, Switzerland, 4 University of Geneva, Switzerland, 5 Cellestia Biotech AG, Hochbergerstrasse 60C, 4057 Basel, Switzerland

DEVELOPMENT OF A NOVEL FIRST-IN-CLASS ORAL INHIBITOR OF THE NOTCH PATHWAY

Abstract:

NOTCH signaling is a developmental pathway known to play critical roles during embryonic development as well as for the regulation of self-renewing tissues. Aberrant activation of NOTCH signaling leads to deregulation of the self-renewal process resulting in sustained proliferation, evasion of cell death, loss of differentiation capacity, invasion and metastasis, all of which are hallmarks of cancer. Over activation of NOTCH in human cancers can be a consequence of over expression of NOTCH ligands/receptors, GOF mutations in NOTCH receptors as well as chromosomal translocations leading to constitutive activation of the pathway.

Given the importance of Notch signaling in human cancers, several therapeutic approaches have been utilized to block NOTCH signaling. Two of these strategies are; a) the use of monoclonal blocking antibodies (Mabs) against NOTCH ligands and receptors and b) the use of small molecule -secretase inhibitors (GSIs). However, these approaches can only be effective if tumor cells express full-length ligand or receptor molecules. On the contrary, in human cancers harbouring NOTCH gene fusion due to chromosomal translocations, the use of Mabs and GSIs will have very limited clinical benefits. A third, yet not fully explored approach

could be the blockage of NOTCH signalling by targeting the most downstream event in the NOTCH cascade i.e NOTCH transcriptional activation complex using small molecule inhibitors. Here we report discovery and identification of a novel, orally-active small molecule inhibitor, named CB-103, of the NOTCH pathway that blocks NOTCH signaling by targeting the NOTCH transcriptional activation complex in the nucleus.
CB-103 has shown the ability to block NOTCH signalling in human T cell acute lymphoblastic leukemia cancer cell lines, induce neurogenic phenotype in drosophila, induce muscle cell differentiation and inhibit NOTCH dependent cellular processes in mice. Furthermore, CB-103 has shown a remarkable activity in ex vivo and in vivo patient derived models of human T-ALL harbouring activation of the NOTCH pathway. In addition, CB-103 exhibit anti-tumor efficacy in a xenograft model of human triple negative breast cancer resistant to GSIs and Mabs against NOTCH ligands/receptors.
Based on in vivo pharmacokinetic/ADME studies, CB-103 has been nominated as development candidate for further preclinical and clinical development.
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Keywords: Notch signaling, T cell acute lymphoblastic leukemia, Targeted

7.00 - 9.00 pm
Welcome Reception Cocktail

Thursday 16 th June 2016

SESSION 2 Chairman: Gian-Paolo Dotto

9.30 - 10.00 am Martin Baron, Faculty of Life Science, University of Manchester

Tuning Notch signalling through an endocytic regulatory network:- revisiting old genetic problems with new insights

There have been 100 years of study of the *Notch* gene following pioneering work on mechanisms of inheritance by Thomas Hunt Morgan. Mutational analysis of Notch subsequently revealed its complex genetics with many different context-dependent alleles exhibiting tissue specific, often temperature sensitive phenotypes whose mechanistic bases are poorly understood. Through computational simulation, cell biology and whole genome RNAi studies, we showed that the setting of Notch signalling levels is deeply embedded in the physiology of the cell through the operation of an endocytic trafficking network that tunes signal activity by regulating Notch flux towards inhibitory or signal activation outcomes. Our new model can explain long standing observations of perplexing temperature and genetic background dependent switching between loss and gain of function Notch signalling phenotypes in *Drosophila* and is providing a new overview to understand the relationship between structure and function of Notch and its mutant phenotypes. The advent of cancer genome sequencing is now providing a huge bank of mis-sense mutations with a similar spread of spread throughout the Notch protein as the classical Notch alleles. We are using this new resource to probe Notch structure function in the Drosophila model to gain insights into possible mechanisms of mis-regulation behind such disease-associated mutations.

10.00 - 10.30 am G. Paolo Dotto, Department of Biochemistry, University of Lausanne, CH

Multistep process of cancer associated fibroblast (CAF) determination under combined CSL-p53 control

The vast majority of epithelial cancers is limited to in situ lesions that, for internal organs like breast, prostate or lung, can remain undetected for the whole life of an individual. The reason(s) why only a minor fraction of these lesions progresses into malignancy is not understood. In fact, many if not most of genetic

changes found in invasive and metastatic tumors can be already present in pre-malignant lesions, raising the question of whether such changes are of primary causative significance or merely permissive for later cancer-spreading events.

Changes in tumor stroma are most frequently viewed as secondary to changes in the epithelium. However, recent evidence indicates that they may play a primary role. Such a possibility would help explain not only dormancy of most epithelial cancers, but also field cancerization, a condition of major clinical significance linked with multifocal and recurrent tumors and broader tissue changes beyond areas of tumor development.

In this presentation, I will overview our recent and ongoing work in this area, with a specific focus on a novel functional and physical cross-talk between the CSL and p53 proteins at the basis of cancer associated fibroblast (CAF) determination.

10.30-11. 30 Coffee Break

SESSION 3 Chairman: Robert Clarke

11.30 - 12.00 George Sflomos[1], Valerian Dormoy[1], Tauno Metsalu[2], Rachel Jeitziner[1], Laura Battista[1], Valentina Scabia[1], Wassim Raffoul[3], Jean-Francois Delaloye[3], Assya Treboux[3], Maryse Fiche[3], Jaak Vilo[2], Ayyakkannu Ayyanan[1] and Cathrin Brisken[1]

[1]ISREC – Swiss Institute for Experimental Cancer Research, School of Life Sciences, Ecole polytechnique federale de Lausanne (EPFL), SV2.832 Station 19, 1015 Lausanne, Switzerland
[2]Institute of Computer Science, University of Tartu, Liivi 2, Tartu 50409, Estonia
[3]Lausanne University Hospital, 1011 Lausanne, Switzerland

Epithelial Microenvironment as Determinant of Luminal Phenotype and Hormone Response in an ERα-positive preclinical model

Seventy-five percent of breast cancers are estrogen receptor positive (ER+). Research on these tumors is hampered by lack of adequate in vivo models; cell line xenografts require non-physiological hormone supplements, and patient-derived xenografts (PDXs) are hard to establish. We show that the traditional grafting of ER+ tumor cells into mammary fat pads induces TGFb/SLUG signaling and basal differentiation when they require low SLUG levels to grow in vivo. Grafting into the milk ducts suppresses SLUG; ER+ tumor cells develop, like their clinical counterparts, in the presence of physiological hormone levels. Intraductal ER+ PDXs are re-transplantable, predictive, and appear genomically stable. The model provides opportunities for translational research and the study of physiologically relevant hormone action in breast carcinogenesis.

12.00 - 12.30 pm Francis Kobia, Victor Alfred, Francesca Carminati and Thomas Vaccari
IFOM, The FIRC Institute of Molecular Oncology,Milan ,Italy

A High Content Screen for Novel modulators of the Notch pathway

Notch signaling is prominently involved in cell fate decision and growth regulation in metazoan tissues. Because of this, Notch is often upregulated in cancer and current efforts point to developing drugs that control its activation in a context-dependent manner. The functions of Notch throughout the life of an individual are complex and not entirely accounted for by the limited core of known Notch signaling components. Interestingly, Notch receptor endocytosis towards acidic compartments is a recently appreciated determinant of signaling activation and a

growing body of evidence indicate that membrane trafficking factors might fine-tune Notch signaling outputs. Considering this, we sought to identify novel genes that might influence localization of Notch receptors. To this end, we performed a high-content RNA interference screen of a pharmacologically-relevant subset of the human genome. Specifically, we monitored which genes knockdowns change the distribution of the endogenous NOTCH1 receptor in human breast cells under resting and signaling conditions. At the meeting, we will present the screen setup, execution and validation and the initial follow up characterization of a subset of the 50 new Notch regulators that we have isolated. Our effort might provide novel entry points for future therapeutic approaches.

1.00 – 3.00 pm **Lunch Break**

3.00 – 4.00 pm **Open Air workshop: Canonical and non-Canonical Notch pathways Barbara Osborne, Gian Paolo Dotto**

SESSION 4 Chairman: Barbara Osborne

4.00-4.30 pm Esther Bridges, Helen Sheldon, Esther Kleibeuker, Evelyn Ramberger, Ulrike Harjes, Ji-Liang Li, Massimo Masiero, Adrian L Harris

University of Oxford Department of Oncology, Churchill Hospital, Oxford, UK

Role of RHOQ induction by Dll4/Notch as an essential mediator of angiogenesis via redistribution of the Notch intracellular domain

Abstract – General

Objective: The Notch ligand Delta-like 4 (Dll4) functions as a negative regulator of angiogenesis. We investigated the role of RHOQ, a member of the Rho GTPase family in mediating the effects of Dll4/Notch signalling during angiogenesis in endothelial cells, as we had found it was highly induced by Dll4 signalling,

Methods and Results: RHOQ was confirmed as a critical downstream mediator of Dll4/Notch signalling using immobilised human recombinant Dll4 (rhDll4) stimulated Human Umbilical Vein Endothelial Cells [HUVECs] *in vitro*. Transient or stable loss of RHOQ expression resulted in excessive angiogenic sprouting and branching and cells were unable to form networks in tube formation assays. Over-expression of RHOQ reduced sprouting and increased sprout lengths, although no change in formed network tube formation was observed. Ability of cells to invade and form networks was disrupted in matrigel *in vivo* plugs containing siRNA duplexes. Stable loss or over-expressing RHOQ caused a significant loss in vasculature formation by P14 of development in the Chicken chorioallantoic membrane [CAM] assays. Retina vessel development and organisation was also

disrupted at P5 following injection of siRNA duplexes targeting RHOQ into neonates on day 3.

Surprisingly, loss of RHOQ greatly reduced expression of downstream targets of Dll4/Notch signalling, whereas over-expression of RHOQ promoted signalling *in vitro*. Cells lacking RHOQ accumulated the Notch1/NICD in the cytoplasm of rhDll4-stimulated cells.

Conclusions: RHOQ was found to have a key role in trafficking the Notch1/NICD from the membrane to the nucleus. Loss of RHOQ expression reduced Dll4/Notch signalling, leading to abnormal regulation of angiogenesis. This work highlights the essential role of RHOQ in mediating Notch signalling and a further 'feed forward' effects of Dll4 signalling.

Abstract – (Max 150words), general breakdown

The Notch ligand Delta-like 4 (Dll4) functions as a negative regulator of angiogenesis (formation of new blood vessels). We investigated the role of RHOQ, a member of the Rho GTPase family, in mediating the effects of Dll4/Notch signalling during angiogenesis. Surprisingly, we found that RHOQ deletion lead to excessive angiogenic sprouting, as well as substantially decreasing the level of Notch signalling. Conversely, over-expression of RHOQ promoted Notch signalling

but also lead to abnormal blood vessel formation. Our results demonstrate that RHOQ plays a central role in mediating and equilibrating Dll4 signalling, acting in a feedback loop that is essential for trafficking the NICD to the nucleus, and has proven to be an essential mediator of the Dll4/Notch regulation of angiogenesis.

Summary

The downstream targets and pathways that are regulated by Dll4/Notch signalling and their subsequent contribution to angiogenesis is not fully understood. We found RHOQ is induced by Dll4 signalling. Silencing RHOQ expression resulted in abnormal sprouting and blood vessel formation both *in vitro* and *in vivo*. Loss of RHOQ greatly decreased the level of Notch signalling, conversely over-expression of RHOQ promoted Notch signalling. A new mechanism regulating Dll4/notch signalling has been found involving Dll4 induction of RHOQ and its subsequent role in trafficking the NICD to the nucleus. Collectively, these results demonstrate the critical role of RHOQ in the finely tuned regulation of angiogenesis by Dll4/Notch signalling.

Significance

The finding that the Rho GTPase, RHOQ has an essential feedback role in Dll4 notch signalling, being induced by Dll4 and pivotal in the transmission of the NICD signal by translocation is a new dimension to notch signal regulation and highlghts the need for further investigation of this family of enzymes in angiogenesis, as well as its assessment as a therapeutic target.

Highlights

- RHOQ is a novel downstream target of Dll4/Notch signalling pathway
- RHOQ plays an important role in angiogenesis *in vitro* and *in vivo*
- RHOQ is involved in trafficking the NICD component of the Dll4/Notch signalling pathway to the nucleus to allow signalling
- Induction of RHOQ is a major positive feed forward loop to induce Notch signalling
- RHOQ activity is critical to maintain the level of Dll4/Notch signalling in endothelial cells

4.30-5.00 pm Helmut Schaider | Associate Professor of Dermatology
Melanoma Biology Group|Dermatology Research Centre | School of Medicine | The University of Queensland
Room 5097, Level 5, West Wing, Translational Research Institute (TRI) | 37 Kent Street | Woolloongabba QLD 4102

The other side of the coin: Notch4 promotes a mesenchymal-epithelial transition in melanoma

The expression of the constitutively active intracellular domain of Notch4 (N4ICD) triggers a mesenchymal-epithelial-like transition in melanoma. N4ICD overexpressing cell lines show an epithelial-like phenotype compared to parental cells. This phenotype is characterized by strongly reduced invasive, migratory and proliferative properties in vitro concomitantly with a down regulation of epithelial-mesenchymal transition markers Snail2 , Twist1, Vimentin, and MMP-2 as well as the re-expression of E-cadherin. In vivo these profound alterations resulted in a significantly reduced tumour growth. N4ICD induced transcription factors Hey-1 and Hey-2 bind directly to promoter regions of Snail2 and Twist1 and suppress gene transcription as determined by EMSA and luciferase assays. Our result indicate that N4ICD overexpression supports a mesenchymal-epithelial-like transition, thus inhibiting the dissemination from the primary tumour mass, trapping the cancer cells in a low proliferative, invasive and migratory epithelial state, suggesting Notch4 to be a tumour suppressor in melanoma. Increased transcript levels of WNT5A in N4ICD overexpressing cells suggest a crosstalk between both pathways. The increase in transcript levels however does not correspond to protein levels as overexpression of Notch4 suppresses Wnt5A. Similarly, overexpression of WNT5A increased transcript levels but decreases protein levels of Notch4 indicating a complex regulatory crosstalk between Notch4 and Wnt5a.

8.00 pm until late Conference Dinner

Friday 17th June 2016

SESSION 5 Chairman: George Sflomos

9.30 - 10.00 am Dimitris Skokos, PhD; Regeneron Pharmaceuticals,Inc.

Delta-like Ligand 4-Notch signaling inhibition regulates pancreatic islet function and insulin secretion

Dll4-Notch signaling pathway is required for cell fate decisions during development and several neoplasias. While Notch antagonists are in clinical testing against malignant transformation, emerging evidence indicates a role for Dll4-Notch signaling in the pathophysiology of metabolic and immune diseases. Along these lines, recent data showed that inhibition of Notch provides an alternative path to modulate FoxO1-dependent gluconeogenesis, as demonstrated by improved glucose tolerance in *L-Rbpj* mice. Further, Notch signaling is proposed as a therapeutic target for type-2 diabetes, liver steatosis and atherosclerosis. However, to date there is no evidence to show direct effects on pancreatic islet function and insulin secretion. Here we identified a missed function of Dll4-Notch signaling inhibition on the biology of insulin-producing

cells. Our findings demonstrate for the first time that administration of anti-Dll4 antibody (anti-Dll4 Ab) targets beta cells in the pancreas to preserve islet functionality, inhibit apoptosis and confer protection from development of streptozotocin and diet-induced diabetes in mice. Further we confirmed expression of Dll4 in pancreatic islets and demonstrated the direct effect of anti-Dll4 Ab in insulin secretion on purified islets *ex vivo*. Importantly, inhibition of Dll4 *in vivo* increased insulin sensitivity by inducing the differentiation of pancreatic beta cell progenitors, and the proliferation of insulin-secreting cells in mice on normal diet. These findings reveal an unidentified, direct effect of Dll4 on pancreatic islets that, in conjunction to its immunomodulatory effects, could provide a new insulin secretagogue for clinical use.

10.00 - 10-30 am Ahmet Acar*, Ana Hidalgo-Sastre*, Giovanna M. Collu, Michael K. Leverentz, Christopher G. Mills, Simon Woodcock, Charles Streuli, Andrew Gilmore, Martin Baron & Keith Brennan

Faculty of Life Sciences, University of Manchester, Oxford Road,Manchester

*** these authors contributed equally**

Should the crosstalk between the Notch and Wnt signalling pathways influence our treatment of cancer?

Notch and Wnt are two essential signalling pathways that help to shape animals during development and adult tissue homeostasis. Although, they are often active at the same time within a tissue, they typically have opposing effects on cell fate decisions. In fact, crosstalk between the two pathways is important in generating the great diversity of cell types that we find in metazoans. In addition, altered signalling through both pathways has been linked to the initiation and progression of human cancer. In this talk, we will explore the molecular basis of these different mechanisms in vertebrate cells and the implications that the crosstalk mechanisms may have on our targeting these two signalling pathways in cancer.

10.30 - 11.30 am **Coffee Break**

SESSION 6 Chairman: **Keith Brennan**

11.30 -12.00 noon **Bruno M. Simões, Ciara S. O'Brien, Rachel Eyre, Andreia Silva, Ling Yu, Aida Sarmiento-Castro, Denis Alférez, Kath Spence, Angélica Santiago-Gómez, Francesca Chemi, Ahmet Acar, Ashu Gandhi, Anthony Howell, Keith Brennan, Lisa Rydén, Stefania Catalano, Sebastiano Andò, Julia Gee, Ahmet Ucar, Andrew H. Sims, Elisabetta Marangoni, Gillian Farnie, Göran Landberg, Sacha Howell and Robert B. Clarke**

Breast Cancer Now Research Unit, University of Manchester, UK

Anti-oestrogen Resistance in Human Breast Tumours Driven by JAG1-NOTCH4-

Dependent Cancer Stem Cell Activity

Breast cancers (BCs) typically express oestrogen receptors (ERs) but frequently exhibit de novo or acquired resistance to hormonal therapies. Here, we show that short-term treatment with the anti-oestrogens tamoxifen or fulvestrant decrease cell proliferation but increase BC stem cell (BCSC) activity through JAG1-NOTCH4 receptor activation both in patient-derived samples and xenograft (PDX) tumours.

In support of this mechanism, we demonstrate that high ALDH1 predicts resistance in women treated with tamoxifen and that a NOTCH4/HES/ HEY gene signature predicts for a poor response/prognosis in 2 ER+ patient cohorts. Targeting of NOTCH4 reverses the increase in Notch and BCSC activity induced by anti-oestrogens. Importantly, in PDX tumours with acquired tamoxifen resistance, NOTCH4 inhibition reduced BCSC activity.

In summary, we establish that BCSC and NOTCH4 activities predict both de novo and acquired tamoxifen resistance and that combining endocrine therapy with targeting JAG1-NOTCH4 overcomes resistance in human breast cancers.

12.00 - 12.30 pm Benedetto Daniele Giaimo & Tilman Borggrefe,
Institute of Biochemistry, University of Giessen, Giessen, Germany
Chromatin Regulation fine-tunes the Notch signaling response

The Notch signaling pathway is a central regulator of differentiation and developmental processes. Aberrant Notch, through Notch1 mutations or dysregulation, contributes to human diseases such as cancer. After specific ligand binding, Notch receptor is cleaved and its intracellular domain (NICD) migrates into the nucleus and associates with the transcription factor RBP-J. In the absence of a Notch signal, RBP-J represses Notch target genes by recruiting a corepressor complex. The switch between repression and activation depends on chromatin regulation at Notch target genes that is achieved via a dynamic recruitment of chromatin modifiers. Here, I will present how dynamic changes in histone acetylation and methylation critically regulate the Notch response and how

signaling may affect chromatin structure. In addition, I will discuss how chromatin modifiers regulate amplitude and duration of the Notch response.

1.00 – 3.00 pm **Lunch break**

3.00-4.00 pm **Open Air workshops:**
Notch Therapeutics Dirk Webber, Adrian Harris

SESSION 7 Chairman: Agamemnon Epenetos

4.00 - 4.30 pm Dimitris Skokos, PhD; Regeneron Pharmaceuticals,Inc.

Next generation of Immuno-therapeutics

4.30 -5.00 pm Adrian Harris, University of Oxford Department of Oncology, Churchill Hospital, Oxford, UK

What does the future hold for Notch Research ?

5.00 -5.05 pm Agamemnon Epenetos - Adjourn to 2017