

EACR Special Session at ECCO-14

With more than 800 EACR members at ECCO-14, the EACR Special Sessions were a particularly attractive feature of the extensive conference programme. Here we present three Special Session reports from Forest White, Joan Seoane and Claus Belka

Network Analysis of Cellular Signalling **Forest M. White**

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Dysregulated cellular signalling networks underlie the development or progression of multiple human pathologies, including autoimmune diseases, metabolic disorders, and cancer. To develop optimal therapeutics to treat these disease states, it is necessary to define not only which components are dysregulated, but also how this lack of regulation affects the biology of the system. In the past, identification of dysregulated components in the signalling network has been performed on a relatively small scale, interrogating the activity or phosphorylation status of selected proteins, and testing their contribution to the biological phenotype. These studies have uncovered much of

the existing knowledge of cellular signalling and have identified many of the existing drug targets. However, this approach to signalling networks is ultimately limited, as it does not yield systems-level information, including cross-talk and feedback between multiple components and pathways. In fact, to understand complex biological processes such as the development of resistance to therapeutic agents, it may be necessary to capture a larger-scale view of the network to identify which compensation mechanisms for inhibition of the targeted nodes. To generate this systems-level view of cellular signalling networks, my lab has developed a mass spectrometry-based technology enabling quantification of hundreds of tyrosine phosphorylation sites across multiple biological samples, all in a single analysis (1).

We have recently applied this technology to U87MG glioblastoma cell lines expressing titrated levels of the constitutively active mutant receptor tyrosine kinase EGFRvIII (2). Quantification of tyrosine phosphorylation sites in these cells revealed a novel cross-talk between EGFRvIII and an activating phosphorylation site on c-Met, the hepatocyte growth factor receptor tyrosine kinase. To test the functional significance of c-Met activation in the EGFRvIII signalling network, cells were treated with small molecule kinase inhibitors targeting c-Met or EGFR, alone and in combination. Although cells were resistant to either inhibitor by itself, treatment with both inhibitors in combination led to a significant decrease in cell viability. Interestingly, treatment with either the EGFR inhibitor or the c-Met inhibitor

sensitized the cells to cisplatin, implicating the c-Met pathway in mediating EGFRvIII-induced chemoresistance in glioblastoma cells. These results highlight the ability of systems-level analyses of signaling networks to identify novel pathways and provide potential therapeutic targets in disease states.

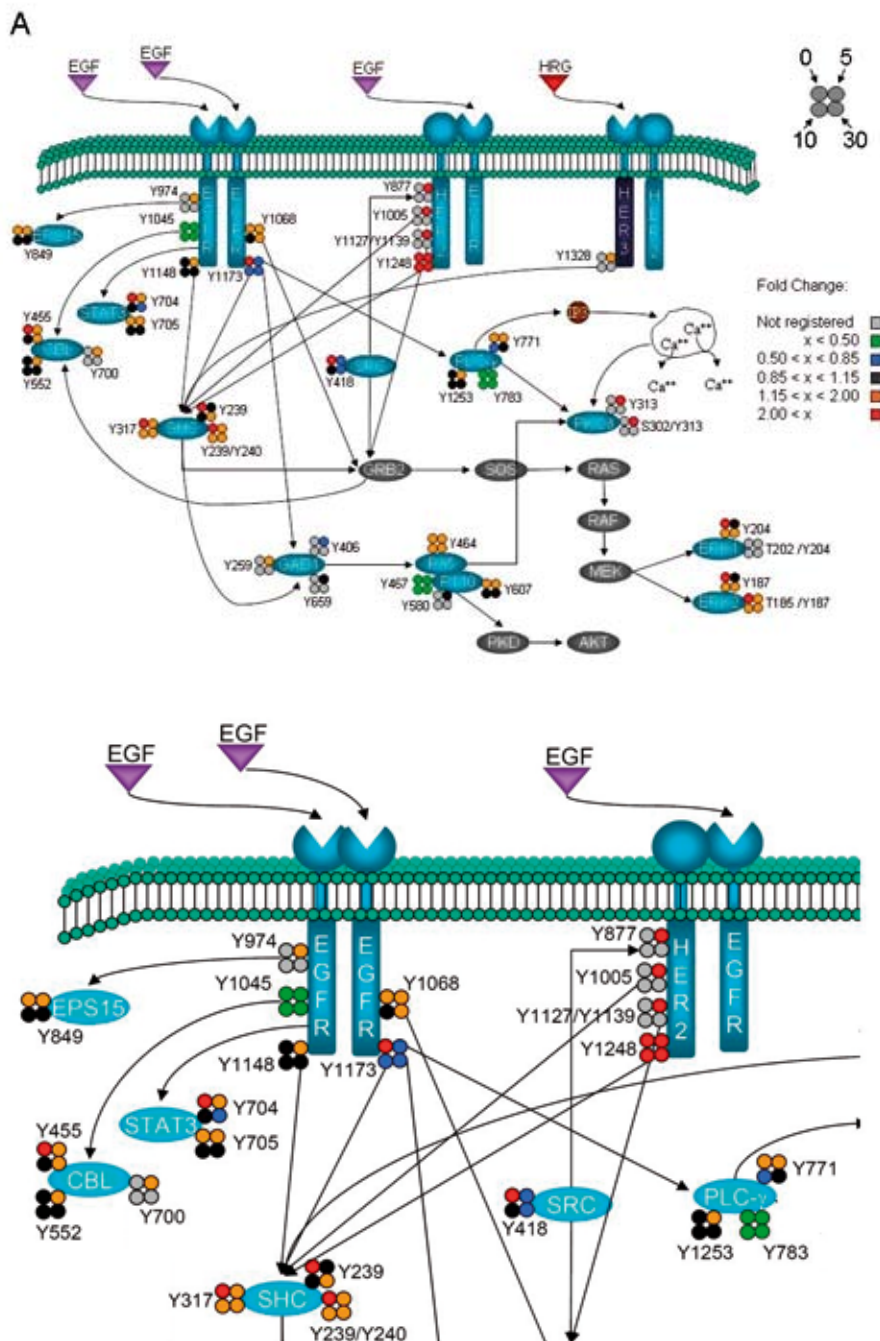
To define how HER2 expression affects downstream signalling networks and resultant biological phenotypes, we have also applied our mass spectrometry-based phosphoproteomic analysis methodology to human mammary epithelial cells with high vs. low HER2 expression levels, in the context of epidermal growth factor (EGF) or heregulin stimulation (3). Temporal profiles were generated for several hundred tyrosine phosphorylation sites across each cellular condition. To parse out the relationship between altered phosphorylation intensity and downstream biological response to stimulation, proliferation and migration data were quantified for these same conditions and a computational model correlating the phosphorylation data to the phenotypic data was generated. This model provides strength of correlation for each phosphorylation site relative to either migration or proliferation, and thereby generated multiple hypotheses that may be tested in the future as to the differential phenotypic effects of inhibiting given phosphorylation events. This study highlights the potential of systems-level analyses to identify novel phosphorylation sites involved in selected signalling networks and assign potential function to these sites, coupling

molecular-level resolution with network-wide analysis.

Systems-level analysis of multiple signalling networks has begun to reveal emergent properties shared across multiple systems (4), and has also highlighted the context-dependent nature of the relationship between signals and biological response (5). Future network-wide analyses will provide additional mechanistic insight into the signals and pathways governing complex biological processes. These insights will enable the development of more intelligent targeted therapeutics to intervene in a variety of human pathologies.

References

1. Zhang, Y., Wolf-Yadlin, A., Ross, P.L., Pappin, D.J., Rush, J., Lauffenburger, D.A. & White, F.M. Time-resolved Mass Spectrometry of Tyrosine Phosphorylation Sites in the Epidermal Growth Factor Receptor Signaling Network Reveals Dynamic Modules. *Mol Cell Proteomics* 4, 1240-1250 (2005).
2. Huang, P.H., Mukasa, A., Bonavia, R., Flynn, R.A., Brewer, Z.E., Cavenee, W.K., Furnari, F.B. & White, F.M. Quantitative analysis of EGFRvIII cellular signaling networks reveals a combinatorial therapeutic strategy for glioblastoma. *Proc Natl Acad Sci U S A* 104, 12867-12872 (2007).
3. Wolf-Yadlin, A., Kumar, N., Zhang, Y., Hautaniemi, S., Zaman, M., Kim, H.D., Grantcharova, V., Lauffenburger, D.A. & White, F.M. Effects of HER2 overexpression on cell signaling networks governing proliferation and migration. *Mol Syst Biol* 2, 54 (2006).
4. Miller-Jensen, K., Janes, K.A., Brugge, J.S. & Lauffenburger, D.A. Common effector processing mediates cell-specific responses to stimuli. *Nature* 448, 604-608 (2007).
5. Janes, K.A., Albeck, J.G., Gaudet, S., Sorger, P.K., Lauffenburger, D.A. & Yaffe, M.B. A systems model of signaling identifies a molecular basis set for cytokine-induced apoptosis. *Science* 310, 1646-1653 (2005).



CARCINOGENESIS AWARDS

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*for major contributions to research
in the field of carcinogenesis*

Winner of the *Carcinogenesis*
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VICTOR VELCULESCU

*for a recent, significant contribution to carcinogenesis
research by an investigator under the age of 40*



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at EACR20,

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Monday 7 July

(13:45-14:35)

2008 Anthony Dipple
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R. Weinberg (US)

EACR-20 Award Lecture

Tuesday 8 July

(12:30-13:15)

2008 Carcinogenesis Young
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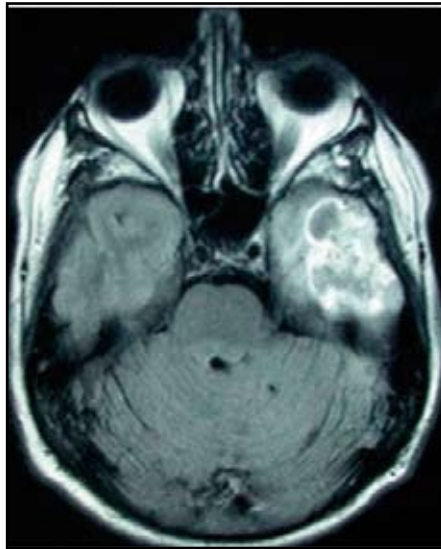
EACR-20 see pages 40-42

The oncogenic role of TGF-beta in glioma

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Glioma is the most common tumour of the brain and its most malignant form, glioblastoma multiforme, is one of the most aggressive human cancers. Treatment strategies of this disease are still barely effective. Despite recent advances in the understanding of the molecular mechanisms that govern glioma, still much more has to be done in order to improve present therapeutic approaches. TGF-beta is a crucial cytokine in tissue homeostasis and embryonic development and has an important role in oncogenesis. TGF-beta acts as a tumour suppressor in normal epithelial cells and early stage tumours, and becomes an oncogenic factor in advanced tumours. The oncogenic role of TGF-beta has prompted the design of several compounds to be used as anti-TGF-beta therapies in cancer. Importantly, the dual role of TGF-beta in oncogenesis presents a unique challenge that has to be addressed to be able to select the patient population that may benefit from an anti-TGF-beta therapy. The understanding of the molecular mechanisms of TGF-beta action and the discovery of predictors of TGF-beta response is required for patient stratification and the development of a successful therapeutic strategy. In some glioma tumours, TGF-beta acts as an oncogenic factor. We have demonstrated that high TGF-beta-Smad activity is present in aggressive, highly proliferative gliomas and confers poor prognosis in patients with glioma. We have discerned the mechanisms and molecular determinants of the TGF-beta oncogenic response using a transcriptomic approach and analyzing primary cultured patient-derived tumour cells, patient-derived glioma stem cells, and human glioma



biopsies. We have identified PDGF-B as the mediator of the induction of proliferation and tumour progression by TGF-beta. PDGF-B is induced by TGF-beta in glioma tumours where the PDGF-B gene is not methylated. Hence, the TGF-beta proliferative/oncogenic response is dictated by the methylation status of the PDGF-B gene. TGF-beta acts as an oncogenic factor and it can be considered a therapeutic target in tumours with an unmethylated PDGF-B gene.

Clinicians and Scientists - No common language?"

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Over the last 20 years cancer research has become an extremely active and complex field. It reaches from cancer prevention and early detection, over cancer diagnosis and staging, to many different facets of cancer treatment. Quite evidently it includes aspects of biology, biochemistry, cell biology, molecular biology, genetics, molecular genetics, pharmacology, toxicology and pharmaceutical technology. However, --and probably less recognized -- important input comes from less obviously related fields like physics, information technology, mathematics, statistics and epidemiology. All these different aspects have their unique input and will undoubtedly influence clinical cancer medicine.

Noteworthy real clinical progress requires an intensive interaction between scientists and clinicians to ascertain a rapid integration of research results into clinical settings. Furthermore a close collaboration between scientists and physicians is of utmost importance in order to keep preclinical research on clinically relevant tracks. Thus, biomedical research in oncology requires a closely integrated and iterative process with involvement of both clinically active researchers as well as basic science experts.

However, in reality, things look different. Currently -- at least to my personal expertise -- many examples of active cancer research groups exist having lost their "clinical counterpart". On the other hand, basic scientists are frequently faced with clinically active counterparts having unrealistic expectations and asking for rapid solutions for quite global questions.

Thus the need for intense and close interaction is clear, however, there is still much room for improvement. Several aspects have to be taken into account when trying to pinpoint the reasons for inefficient interaction between basic scientists and clinicians. For me, one of the most evident aspects is related to the general attitude of the professions involved. In other words, the driving force behind a doctor in daily clinical routine differs strongly from what makes a scientist move. Basically, the physician is faced with a -- in case of cancer- human being suffering from a potentially deadly disease. Thus an immediate action is required.

On the other side, the scientist is faced with a theoretical problem or an observation which he wants to understand. However, in no case an immediate action is required. In other words physicians are requested to come up with mature decisions within in a short time. In cancer medicine, decisions are frequently irreversible and may have tragic consequences. The decisions are mainly based on experience integrating knowledge and intuition. A wide variety of so called human factors have to be integrated. The whole process of decision making

is driven by the need to come to a solution. Thus complex problems have to be scaled down and condensed. Minor aspects have to be ignored and some kind of gut feeling is allowed.

In sharp contrast, a scientist starts with time for a precise definition of a given problem. During work errors are allowed and also required to generate new hypotheses. The general approach is driven by scientific facts and all decisions during a scientific process are --more or less -- "evidence" based. The influence of "human factors" can be minimized and the whole process is cognition based.

Closely related to the field of attitudes and driving forces completely different perceptions about time frames exist. Whereas the physician asks for a solution now -- the real research chain from basic research to a clinical application entering a phase I trial takes years. In my experience the magnitude of these time lines is frequently underestimated by many clinicians. Next to different driving forces and approaches to problems, the use of a highly specialized terminology is an obstacle for fruitful interaction. Although most medical and biological backgrounds should be easy to comprehend by any academically trained individual in the field of biomedicine, technical terms and acronyms hamper an easy access to certain research fields. In my personal experience, many clinically active physicians are discouraged simply by the use and the number of acronyms. The same is true for physicians who --in a way-- "hide" behind a number of latinisms.

For me, overcoming those fundamental differences in attitude and language are the most important determining factors for fruitful interactions. In other words both partners have to have very open minds to achieve a solid basis for a mutual understanding. I am strongly convinced that this kind of open mind complemented with adequate know how can only be created during a well designed academic training process. Therefore, in the future structural changes of the academic training process will be required to

cope with these needs.

In the end one would have to create training structures for those who are heading for a "pure" physician training only. Different structure are required for physicians who are also willing to be active in research and finally for those heading for "pure" basic biomedical sciences only. A theoretical training programme is depicted in figure 1.

Taken together, progress in cancer treatment will require a continuous and effective interaction of research partners coming from a wide variety of biomedical subspecialties. In order to facilitate an efficacious collaboration all partners have to be aware of the know-how, the attitude and the respective approach to problems of any of the partners involved. In my opinion, this can only be achieved by structural adaptation during academic training for all those being involved in biomedical research

Thus achieving this goal will be a future challenge for most academic institutions in Europe.

Figure 1
Training in biomedical sciences:

A: Training for a "pure clinician". In contrast, those willing to be active in research start with a more or less complete Bachelor in "Biomedical sciences". Those willing to do only research may complete their studies with a Master degree in biomedical sciences. However, those who are willing to act as physicians, but with a strong involvement in research, could complete their training with the same clinical part as the pure "physicians".

B: After graduation, MD/PhD programmes would allow further qualification with highly trained "Research Medical Doctor" or "Research Medical Scientist" as final "product".

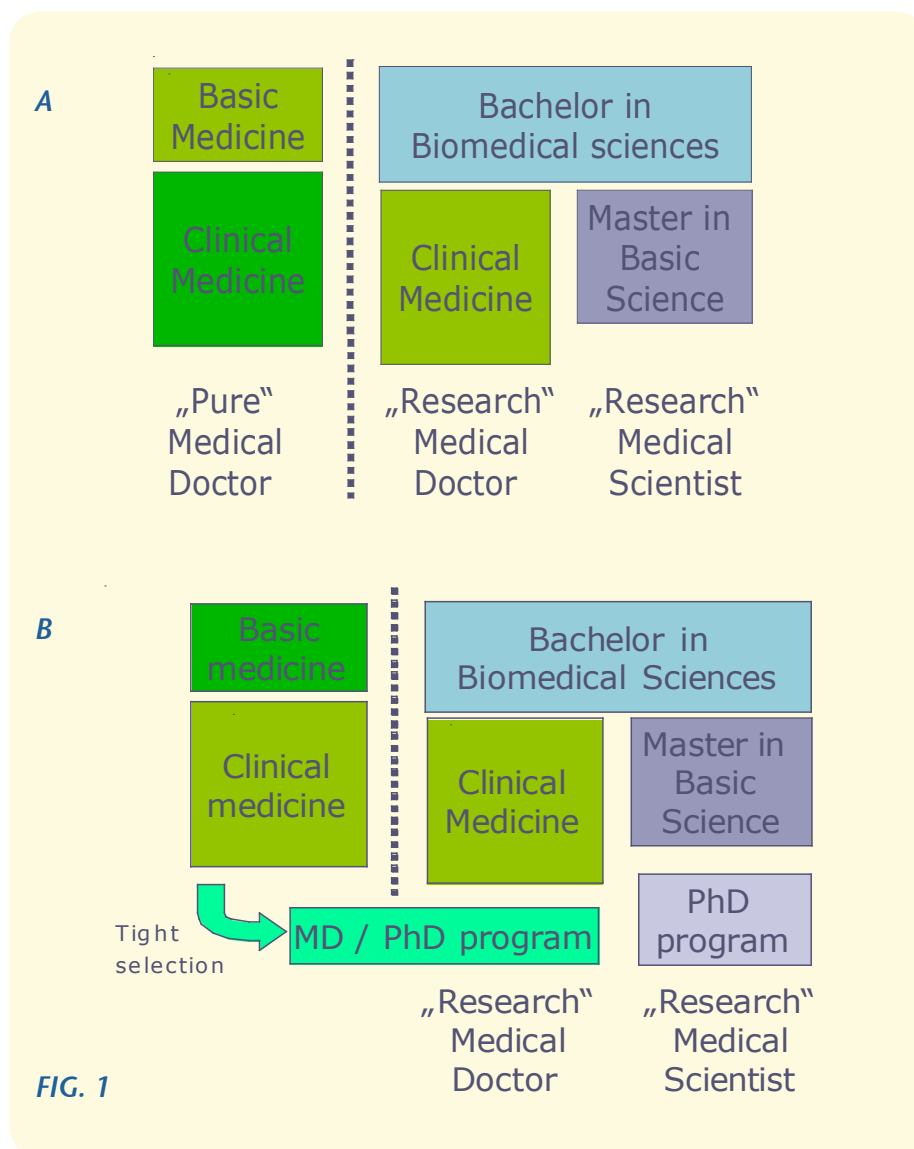


FIG. 1