

EACR-20: The European Translational Oncology Meeting

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Abstract

The 20th biannual Meeting of the European Association of Cancer Research EACR-20 was held in Lyon, in July 2008. The Meeting successfully gathered researchers from basic, translational and clinical cancer research to exchange and discuss recent results in the areas of major interest. This review presents a meeting report and intends to be a portrait of the excellent research presented at EACR-20.

Introduction.

From Saturday July 5th to Tuesday July 8th, EACR-20 welcomed 1151 participants, including 353 students, to the Cité Centre de Congrès Lyon. The Meeting followed The Integrative Molecular Cancer Epidemiology-An IARC-EACR-AACR-ECNIS Symposium from the 3rd- 5th of July. M. A. Pierotti Conference and Scientific chairman together with R.J. White, chairman of the Young Cancer Researchers' Committee and G. Lenoir chairman of the National Organizing Committee, proposed an excellent scientific programme with plenary lectures, parallel scientific symposia, presidential sessions and educational lectures, covering the areas of current interest and the most recent discoveries in cancer research.

Following the success of the initiative at EACR-19, the Young Researchers' Scientific Committee organized two workshops focused on interesting issues for young researchers at the beginning of their scientific careers. These workshops covered strategies for fellowship applications and an introduction to pharmaceutical research and development.

The location of the Cité Centre de Congrès set in attractive surroundings and the hospitality of our hosts in Lyon contributed to a very enjoyable Meeting. The highlight of the social events was the Conference Dinner at Lyon's City Hall, a magnificent building with an exquisite atmosphere. This was the most successful of the EACR Meetings, both for the scientific level and the major interest that the participants demonstrated in high attendance at all sessions. This success was possible due to the contributions made by the local committee, together with the work of the organizing team at ECCO and the EACR Secretariat at Nottingham; to all of them we wish to express our thanks and recognize their effort in making this possible.

Mühlbock lecture

The opening lecture is named after one of the founding members of the EACR, and its First President, Professor Otto Mühlbock and was delivered by Hans Clevers (Utrecht, The Netherlands). He gave a lecture on Identification of stem cells in small intestine by single markers. In an introduction he exposed the current models that indicate that 4-6 crypt stem cells reside in the +4 position to paneth cells in the small intestine while colon stem cells remained undefined. In a screen of Wnt target genes with restricted crypt expression his group identified the Lgr5/Gpr19 gene. By using knock in experiments they established that this gene marks cycling crypt base columnar cells at the crypt base. By tracing in adult mice, they also found that Lgr5 crypt base columnar cells generated all epithelial lineages over a 60-day period, suggesting that it represents the precursors of the small intestine and colon. Furthermore, by studying other tissues it seems that Lgr5 marks stem

cells in multiple adult tissues (stomach, mammary gland, liver, retina, brain) and cancers.

Symposium: Receptor Signaling

The first two talks in this symposium concerned the Epidermal Growth Factor Receptor family as a complex system. Bill Gullick focused on a computer simulation of the receptors and their ligands in which the number of molecules could be varied and their interactions determined. He described an experimental analysis of the complete family in one hundred cases of breast cancer using immunohistochemical staining and reported that there was a strong correlation between expression of each ligand and receptor. In the future this data will be analysed using the simulation to assess the activation state of each receptor to aid in predicting which signal transduction inhibitor would be most effective in individual patients.

Joseph Yarden extended this concept to consider the downstream events elicited by activation of the system including early induction of gene expression and subsequent waves of inhibitory responses. He described how the system had evolved to tolerate and adapt to mutations by the alternate use of intracellular pathways and how this was relevant to the use of individual and combinations of signal transduction inhibitor drugs. He described the plasticity of the system, for instance by variation of receptor downregulation and internalisation, and how targeting multiple pathways would be more likely to be effective therapeutically.

The second pair of talks reviewed the clinical data on signal transduction inhibitor drugs directed at individual receptors or receptor combinations. Nicola Normanno discussed the results to date of small molecule tyrosine kinase inhibitors. Some patients, notably those with mutations in the kinase domain of the EGF receptor, achieved significant responses but many other patients were unresponsive. He described the emerging knowledge of the mechanisms of development of drug resistance and how early studies using combinations of drugs, for instance EGF receptor inhibitors with aromatase inhibitors in breast cancer, may give more frequent and sustained tumour regression. Fortunato Ciardiello gave a complementary talk on the use of monoclonal antibodies to the EGF receptor and the clinical experience to date in the treatment of colorectal cancer. Again a minority of patients responded but it had become clear from several recent studies that this subgroup could be efficiently selected in advance by molecular analysis of their tumours. Here mutations in Ras genes and alterations in the PTEN/PI3kinase pathway accurately predicted patients who would not benefit from this type of treatment.

Overall the symposium emphasized that the EGF receptor system was complex and had evolved to tolerate acquired mutations and that this had implications in therapy strongly suggesting that targeting multiple pathways would be more effective and less prone to evoke drug resistance. Second, there are tests emerging which will allow the selection of patients most likely to respond to available drugs making their use more cost effective.

Symposium "Gene expression and cancer".

Abnormal gene expression is a feature of all known malignancies. This symposium explored several disparate aspects of the gene expression field, each with relevance to cancer. It was opened by Ingrid Grummt (German Cancer Research Centre, Heidelberg), who spoke about the regulation of rRNA synthesis. The large rRNAs that perform structural and catalytic roles in the ribosome are synthesized by RNA polymerase I (pol I) as a long precursor that is then processed into the mature products. Overexpression of this pre-rRNA is a general feature of cancer and may be useful as a prognostic marker. The genes encoding pre-rRNA are found in repetitive clusters. Humans have ~400 individual copies, but only half of these

appear to be active. The remainder are silenced by intriguing epigenetic mechanisms, which were described by Dr Grummt. She has shown that the chromatin remodeling complex NoRC is responsible for switching off half the copies of the pre-rRNA genes, a process that involves methylation of promoter DNA and histone deacetylation. Recruitment of NoRC to these sites requires a short processed transcript of 150-300 nt called pRNA, that is derived from an intergenic site ~2kb upstream and matches the pre-rRNA promoter. The level of pRNA is decreased in some malignant cells, which may release pre-rRNA templates from silencing and allow increased pol I output.

Robert White (Beatson Institute for Cancer Research, Glasgow) described the connections between cancer and RNA polymerase III (pol III), which makes short untranslated RNAs, such as tRNAs. Transformed cells generally overexpress pol III products. To address the significance of this overexpression, cell lines were constructed in which pol III transcription can be increased selectively by induction of the pol III-specific transcription factor TFIIIB. This was found to increase cell proliferation and cause oncogenic transformation. Conversely, depletion of TFIIIB inhibits transformation. Amongst the gene products induced by TFIIIB is the tRNA_i^{Met} that initiates polypeptide synthesis. Overexpression of this tRNA is sufficient to stimulate proliferation and oncogenic transformation by a variety of criteria, including tumour formation in mice. Dr White described some of the mechanisms that can stimulate tRNA production in transformed cells. These include activation of pol III transcription by c-Myc and several oncogenic kinases, which bind directly to TFIIIB. In healthy cells TFIIIB is bound and repressed by p53 and the retinoblastoma protein RB, which restrain pol III output. TFIIIB can also be inhibited indirectly by PTEN. The function of PTEN, p53 and/or RB is compromised in most cancer cells, releasing TFIIIB from restraint and thereby allowing abnormal increases in pol III products, at least one of which, tRNA_i^{Met}, can have proliferative and oncogenic effects.

Qualitative and quantitative changes in protein synthesis are probably responsible for the transforming effects of tRNA_i^{Met}. Protein synthesis also requires the translation initiation factor eIF4E, which is found at elevated levels in many cancer types, including breast, head and neck carcinomas. High expression of eIF4E can transform cultured cells and animal models and correlates with poor prognosis in patients. A subset of mRNAs with complex 5'-untranslated regions are translated preferentially following an increase in eIF4E or tRNA_i^{Met}. This category includes mRNAs for tumour-promoting products, such as c-Myc and cyclin D1. In addition to its well-characterized role as a translation factor in the cytoplasm, eIF4E is also found in the nucleus. Katherine Borden (University of Montreal) demonstrated how the nuclear function of eIF4E contributes to its transforming activity. She explained that eIF4E binds to a sequence motif in the 3'-untranslated regions of certain mRNAs to promote their nuclear export. In some cases, these are the same mRNAs that are preferentially translated in response to cytoplasmic eIF4E, so that two key steps in the production pipeline are stimulated. Dr Borden described how the motif recognised by eIF4E provides an example of a USER code module that is shared by mRNAs involved in a common function, allowing their regulation to be coordinated. For example, eIF4E co-regulates mRNAs for components of the Akt pathway that promote cell survival.

Dove-tailing nicely with Dr Borden's description of control modules at the mRNA level, Antonio Lavarone (Columbia University, New York) described a transcriptional module that synergistically regulates the mesenchymal phenotype of malignant gliomas, which he discovered using a systems biology approach. A mesenchymal gene expression signature is observed in high-grade glioma, but not in normal neural tissue, and represents a hallmark of tumour aggression. Over 74% of the signature genes are regulated by a small module containing six transcription

factors. Two of these factors, Stat3 and C/EBP, display features of initiators and master regulators of mesenchymal transformation. Thus, ectopic expression of Stat3 with C/EBP is sufficient to suppress genes associated with the normal neuronal state and reprogramme neural stem cells towards the aberrant mesenchymal lineage, promoting migration and invasion. The mesenchymal signature collapses and tumour aggression is reduced if Stat3 and C/EBP are silenced in glioma cell lines. In human glioma samples, combined expression of these two transcription factors correlates with mesenchymal differentiation and strongly predicts a poor clinical outcome. Dr Lavarone concluded that a small transcriptional module is necessary and sufficient to reprogramme neural stem cells towards a transformed mesenchymal state. He has therefore succeeded in identifying master transcriptional regulators from human cancer signatures by employing a powerful, experimentally validated computational approach.

Symposium: Diagnostic and predictive molecular markers

The symposium was aiming at discussing recent findings from expression, methylation and mutation analyses of both healthy tissue, premalignant and tumour tissue for development of diagnostic and predictive molecular markers.

In the first talk by AL Børresen-Dale, from the Institute of Cancer Research in Oslo, expression profiling using peripheral blood cells (PBCs) from women diagnosed with breast cancer as well as healthy controls was presented. A gene signature of approximately 100 genes was identified that was differentially expressed between cases and controls with a specificity and sensitivity around 85%. Results from four different studies were presented including an interim report from a larger study of women from India that supported the findings in the other cohorts. The conclusion was that breast cancer affects gene expression patterns in PBC during early stage of disease development, and that the signature identified was independent of ethnicity. Blood expression as a surrogate for early disease detection might be a valuable addition to mammographic screening in particular in women with mammographically dense breasts where mammography is difficult and inconclusive.

In the second talk by P Cairns from Fox Chase Cancer Center in Philadelphia methylation profiling of tumour cells in tissue biopsies, urine, blood and other body fluids was addressed as a promising tool in diagnostics and prognostication of cancer. The average total number of genes methylated with functional significance in a tumour cell is unknown but a reasonable estimate might be several hundreds. Moving from a small set of genes to more global screens elucidating the cancer cell methylome will extend and improve current panels of genes used for early detection. Such new panels may provide signatures for differential diagnosis using body fluids and may also be used in prognosis evaluation. The speaker discussed various challenges for methylation-based detection, like optimization and standardization of specimen processing, technology to be used, and knowledge of timing of methylation of a gene with regard to clinically significant disease, and the ability for differential diagnosis of the anatomical site of origin of a tumour in a body fluid.

In the talk by C Perou from UNC Chapel Hill various expression profiling classifications of breast tumours were presented and discussed in relation to responsiveness to neoadjuvant chemotherapy and tamoxifen. In addition to the intrinsic subtype classification, the MammaPrint™ 70-gene profile and the 21 gene Oncotype DX™ used in the Genomic Health Recurrence Score were evaluated. Within the Basal-like, Luminal B and HER2+/ER- tumour subtypes, great concordance across all predictors was observed, although within the Luminal A group, there was heterogeneity in predictions, suggesting that further stratification is needed. A subgroup of ER negative therapy resistant basal like tumours

designated "Claudin-low" was identified that had an expression signature similar to TIC/stem cells and metaplastic carcinomas. Additional gene expression predictors are therefore needed to guide current therapeutic decision making. He concluded that the best patient treatment is guided by a linked combination of conventional pathology and genomic biomarkers.

In the last talk given by M Olivier from IARC in Lyon the role of somatic TP53 mutations and cancer prognosis was discussed. More than 2000 distinct point mutations have to date been reported in cancer. The IARC database compiles data on cancer specific and population specific distribution of TP53 mutations, and has information about functional impact and prognostic/predictive value of the different type of mutations. It was demonstrated that different missense mutations have different prognostic value. Data from both breast and lung cancer were presented. Convincing data concluded that mutations analyses of TP53 at the sequence level are an important tool in evaluating prognosis and in predicting cisplatin-based chemotherapy.

Plenary Lecture: Breast Cancer stem cells.

This lecture was given by Kornelia Polyak (Boston, USA) who introduced the concept of cancer stem cells and the biological evidence that supports the existence of such cells in breast cancer. They characterize cells with stem-like characteristics by using the markers CD24 and CD44. Gene expression profiles, SNP analysis indicated that CD24+/CD44- and CD24-/CD44+ cells from the same tumour are clonally related, but can be genetically and epigenetically distinct, an example of that is the response to TGF- β signaling pathway. Another interesting observation indicated that CD24+ cells are more abundant in metastasis to distinct organs, even if in the primary breast tumour was enriched in CD44+ cells. This indicated that expression of these markers changes during progression to metastasis or that CD24+ cells are more prone to metastasis. The evidence presented indicated that tumour progression is a continuous selection process, that is strongly influenced by the genetic instability of a tumour in a given time and location.

Symposium Scientific Symposium on Cell Death Pathways

The scientific symposium on cell death pathways was chaired by Verena Jendrossek (Essen, Germany). The first two talks covered diverse aspects of ongoing research on the role of apoptosis in tumorigenesis and anticancer therapy. The role of the Myc-oncogene in tumour development was discussed by Gerard Evan from the University of California San Francisco (UCSF)/Helen Diller Family Comprehensive Center (San Francisco, USA). He first outlined the dual face of this oncogene that drives proliferation but disposes at the same time of inherent tumour suppressor activities triggering p53-dependent apoptosis and senescence. They developed a mouse model that allowed a switch between p53 wild-type and knockout state by addition or removal of 4-OH-tamoxifen, respectively (p53ER^{TAM}). In the Em \square Myc model for lymphoma with knock-in gene replacement of one allele of the endogenous tumour suppressor p53 by p53ER^{TAM} they could demonstrate that activation of Myc fosters inactivation of the remaining p53-allele by deletion or point mutation, recapitulating events observed during Myc-driven tumorigenesis. Tamoxifen-mediated reactivation of the silent p53 wild type allele in a permanent or transient manner triggered either massive necrosis associated with death of the mice by tumour lysis syndrome or extension of lifetime, respectively. He then moved to a mouse model of lung cancer driven by oncogenic Ras. In those tumours Myc functions downstream of Ras and suppression of Myc-transcriptional activity led to tumour regression. Together with the observation that in normal tissues inhibition of Myc caused only arrest of the cells these observations point to a putative role of Myc as target for anticancer treatment.

Andreas Strasser from the Walter and Eliza Hall Institute of Medical Research in Melbourne, (Parkville) Australia discussed the role of the BH3-only protein Bim for apoptosis regulation in vascular endothelial cells. Sustained angiogenesis is critical for tumour growth and involves VEGF-triggered survival and proliferation of endothelial cells. Moreover, therapy-induced regression of tumours is associated with massive endothelial cell death. Since pro- and antiapoptotic members of the Bcl-2 family regulate programmed cell death of normal tissue cells during development and death in response to cellular damage he studied the regulation of endothelial cell death during therapy-induced tumour shrinkage with a focus on BH3-only proteins. Interestingly, in B16F1 melanoma and Lung Lewis carcinoma tumour models loss of Bim in the host endothelial cells inhibited therapy-induced apoptosis resulting in decreased therapeutic efficacy and a more rapid tumour regrowth. These novel findings point to a critical role of endothelial cell survival in tumour regrowth and progression.

The second part of the symposium dealt with the role of autophagy in cancer. Autophagy represents a major homeostatic mechanism for the generation of energy from protein catabolism on the one hand and removal of damaged organelles, respectively. Kevin Ryan from the Beatson Institute for Cancer Research, Glasgow, UK, discussed the autophagic regulation of tumour cell viability. He first introduced the role of DRAM (damage regulated autophagy modulator) in the regulation of the cellular response to stress conditions. Subsequently, he reported about the results of a RNAi-mediated screen in *Drosophila* for specific kinases involved in regulation of autophagy. In further analyses he and his group identified PDGFR kinases to be specifically involved in the hypoxia-mediated autophagic pathway in humans being responsible for the determination of the HIF-1 transcriptome.

The talk of Eileen White, University of New Jersey, Piscataway, USA, on the dual role of autophagy in cancer was moved to the session on „Cancer cell metabolism“, held on Monday afternoon (chaired by Jacques Pouyssegur, Nice, France). As the same molecular alterations that lead to tumour cell survival during tumorigenesis can contribute to resistance to therapy-induced cell death, an improved understanding of the underlying molecular mechanisms is a prerequisite for the development of effective therapeutic strategies to target death pathways in cancer.

Symposium. Mouse Models

Anton Berns (Amsterdam, Netherlands) discussed how insertional mutagenesis screens in KO and wild type mice are useful to identify genes that collaborate with loss of i.e. p19 arf in tumorigenesis. By using a large-scale retroviral insertional mutagenesis high-throughput screen in mice p53^{-/-} or p19 (ARF) ^{-/-} he reported the identification of a high number of new candidate oncogenes and tumour suppressors. He exemplified that Flt3 is mutated in p53 and p19ARF and not in wild type tumours. Furthermore, mapping interaction networks between common insertion sites (CISs) showed that mutations in the Ras-activating Rasgrp1 are mutually exclusive with mutation of Flt3. He presented the possibility that insertions disrupt and inactivate tumour suppressor genes, i.e., Ikaros (Zfpn1a1) harboring 50 insertions. Although, it is possible to identify insertions distributed in clusters that mutated the same gene by different mechanism as he reported for notch1 and Flt3, giving an idea of other control elements in the mechanism of oncogenicity as different CIS could have different synthetically lethal with other pathways. In collaboration with J. Mattsson (Wellcome Trust Sanger Institute's cancer Genome Project), they did a comparative analysis with their CIS loci and a CGH data of human cancer cell lines, finding known and candidate oncogenes and tumour suppressors (Wwox and Arfrp2). This type of analysis is a promising strategy to elucidate resistance mechanism evoked by chemotherapy.

Due to genome instability and heterogeneity of human tumours it is difficult to identify cancer genes and therapeutic targets. During the last two years many efforts have been made in order to integrate cancer genomics and mouse models in order to accelerate the discovery of new cancer genes. Lars Zender (Braunschweig, Germany) discussed how their laboratory developed a "mosaic" mouse model whereby liver carcinomas can be rapidly produced with different genetic alterations. Using this model they identified cIAP1 and Yap as new oncogenes in human hepatocellular carcinoma, while NEDD9 was identified as a metastasis gene in human melanoma. He described how the combination of this mosaic model and RNAi validate DLC1 as a potent tumour suppressor gene. Through using ROMA analysis he demonstrated the discovery of new tumour suppressor genes, that were never before related with cancer, as eIF5A.

S. Aznar (Barcelona, Spain) discussed the mechanisms by which Rac1 controls the epidermal stem cell homeostasis and that regulation. He proposed a model in which human polycomb protein, hPc2 increases activity of Rac1 and as a consequence phospho-cMyc increase, these allow cells to exit from the niche, and terminal differentiation. On the contrary Myc sustained could conduct to hyperproliferation, genome instability and cancer. Human polycomb protein, hPc2 (Cbx4) is a member of PRC1 complex (Cbx4) and cMyc is able to bind to Polycom2. He demonstrated that decreases hPc2 induces irreversible terminal differentiation and hPc2 prevents activation of Stem cells and differentiation.

L. Chin (Boston, USA) discussed about Integrative comparative oncogenomics of mouse and human tumours. Cancer genomics has provided huge genomic data sets that described alterations in DNA sequence or structure, genes or chromosomes. Also cancer cells could contain epigenetics modifications. The question to resolve now is how could we decipher or distinguish between the real modification versus "noise" changes. By using cross-species triangulation with tumour associated alterations in refined genetically engineered mouse models, she compared the data from primary melanoma versus metastatic melanoma and described an important role of the gene HOXA-1 in the metastatic progression.

Plenary Lecture Z. Herceg "Epigenetics and epigenomics" (instead of C. Thompson "Metabolism") Zdenko Herceg from the International Agency of Research in Cancer (Lyon, France) gave a plenary lecture on Epigenetics. By way of introduction, he outlined the observation that in addition to genetic changes that are characteristic for tumour initiation and progression, epigenetic changes also contribute to the malignant process. Histone acetylation and methylation constitute heritable epigenetic alterations that cause changes in chromatin structure and gene expression with relevance for cancer cell signaling and function, e.g. silencing of tumour-suppressor genes, activation of oncogenes and defects in DNA repair. In this scenario, epigenetic changes can be a consequence of genetic alterations and vice versa, changing our common view on the causes of cancer predisposition, initiation and progression. In the second part of his talk he focused on the role of one component of histone acetyltransferase-complexes, the Transformation/transcription domain-Associated Protein Trapp. He summarized the current view on the function of Trapp in DNA repair and the implication of altered Trapp signaling for tumour development.

In the last part of his talk he gave insight into several epidemiologic studies that have been designed to analyze the impact of environmental and dietary agents as well as lifestyle on the occurrence of epigenetic alterations associated with specific human cancers. These should allow a better understanding of the relationship between exposure to known cancer risk factors such as nicotine and epigenetic changes. The development of novel technologies for sensitive and quantitative detection of epigenetic changes as well as for genome-wide analysis was mandatory

to address these issues. A better understanding of the molecular events underlying epigenetic changes during tumorigenesis and malignant progression may help to develop innovative strategies for prevention and treatment of cancer.

Symposium: Invasion and metastasis

M Frame (Glasgow, UK) showed the relationship between the Src/FAK pathway and cancer invasion and metastasis. The Src/FAK pathway is involved in invasion and migration acting over the podosome/invadopodia formation and degrading the extracellular matrix. In addition, FAK is also controlling the adhesion dynamics in cell and therefore, cell migration. Using a mouse skin carcinogenesis model that combines DMBA with TPA they found the expression of FAK to be necessary for the progression of the cancer invasion. Then, Dr. Frame explained that the FAK mechanisms of action involve two complex formations: Actions mediating ARP3 complex: Two regions in FAK that can bind Arp3, inducing a transient complex that promotes the spreading, as it is inhibited after mutating those residues. Actions mediating RACK1 complex: This complex affects the Golgi and cell polarity as mutation of the amino acid involved in the complex formation reduces the binding, cells become round and do not induce polarity and pseudopodia. Dr Frame suggested that Src inhibitors could be used as anti-invasive agents: Src deregulate the E-Cadherin tumour suppressor protein. The use of the Src inhibitor AZD0530 restored the E-Cadherin and inhibits the 2-D planar migration (Wounding healing). Dasatinib also decreased tumour metastasis in a GFP mouse model for pancreatic cancer that can be used to detect early micro-metastasis diseases. Photobleaching and photoactivation are two useful techniques that can provide information about drug-development.

Dr R Klemke (La Jolla, USA) presented cell metastasis as a dynamic process that occurs in multiple steps that include invasion, intravasation, survival in the circulation, extravasation and cell growth at the distant site. Active and passive intravasation is one of the early events involved in this process, being a main issue of study by Dr. Klemke's group. They use a GFP vascular transgenic zebrafish as an animal model to study tumour induction, angiogenic progression and new vessels formation. This model also allows the study of RhocC and VGFC contributions to metastasis. VEGF signaling is necessary to maintain the angiogenic vasculature inducing blood-vessels, while RhocC is contributing to cell invasion through an amoeboid movement and intravasation during early events of cancer metastasis but not in tumour formation. RhocC also plays a role as a late acting metastatic gene that operates after the angiogenic switch has been triggered. Dr Klemke concluded that both, RhocC expression and VEGF secretions cooperate to mediate membrane protrusion into the lumen and intravasation through the induction of primitive amoeboid movements and permeabilization and remodeling of tumour vessels.

Dr Friedl (HB Nijmegen, The Netherlands) combined 3D collagen lattices and in vivo microscopy to study the migration process of cancer cells during invasion. They observed high diversity of cellular aggregation, like individual cells, Indian files or different clusters. Cells get collected and polarized to show less contact with the ECM. To be able to migrate in-group, cells need collagenolysis to degrade the collagen matrix, if proteases are inhibited, then, only individual cells migrate. They also observed that cells are able to divide while they are moving, which have been called "invasion growth". In summary, Dr Friedl reconstructed the subcellular proteolysis during the migration process, the remodeling of the extracellular matrix and the invasion mechanism.

Finally Dr B. Geiger (Rehovot, Israel) (addressed the molecular interactions of cells with the ECM focused on the nature and diversity of the integrin adhesion

machinery according to Complexity, functional dissection, interactions switches and their role in metastasis according to associated pro-migratory-genes.

Symposium: DNA Damage and genome instability

Dr. J.H, Hoeijmakers (Rotterdam, The Netherlands) introduced the mechanism of DNA repair by excision repair focusing in the Nucleotide Excision Repair system (NER). NER deficient patients show basically three different phenotypes X. pigmentosum (7 genes), Cockayne syndrome (CS 5genes) and trichothiodystrophy (TDD) (3 genes), one difference between these diseases is that X.P develops cancer high frequency. Different mutations on the same gene XPD could cause the three symptoms, XP, CS and TTD. Using mouse model systems he described how XPD mutations act as a crosstalk between aging and cancer. XPD/TTD mice, harboring a mutation in the XPD gene (R722W), display severe symptoms of premature aging but have a reduced incidence of cancer. He used comparative microarray analysis of young and old female livers to discover gene expression signatures distinguishing XP/TTD mice from their age-matched wild type controls. They found IGF-1 as a modulator of life span, and while the increased apoptotic response to endogenous DNA damage contributes to the accelerated aging phenotypes and the reduced cancer incidence observed in the XpdTTD mice, the signature of reduced energy metabolism is likely to reflect a compensatory adjustment to limit the increased genotoxic stress in these mutants.

SJ.Kron (Chicago, Il, USA) exposed the aim of his studies conducted to discover the kinetics and molecular determinants of protein localization to double strand breaks and elucidate the consequences of disrupting protein recruitment. Using *S. cerevisiae* as a model system, he discussed how phosphorylation of H2A by ATM homologue Tel1 promotes Rad9 recruitment and checkpoint activation. By obtaining mutations along Rad9 protein this group discover that tudor domains tether Rad9 to Dot1-methylated histone H3 while BRCT domains recognize phosphorylated H2A. Also he has developed a model combining imaging and proteomic analysis of DNA damage foci in cancer cells using fluorescent protein fusions to the checkpoint signaling protein 53BP1. He demonstrated that disruption of 53BP1 chromatin association could acts as a route to radiosensitivity.

Jessica Downs (Brighton, UK) nicely explained how DNA double strand breaks (DSBs) are potentially serious chromosomal lesions. Eukaryotic DSBs that occur in the context of chromatin and the RSC chromatin-remodeling ATPase complex have been shown to promote DSB repair at the budding yeast MAT locus DSB. The RSC complex therefore has chromatin remodeling roles both before and after DSB, promoting both DNA cleavage and subsequent repair - also, explaining that in the orthologue of RSC in mammalian cells is PBAF.

Tony Hazalonetis (Geneve, Switzerland) started his talk discussed common features of precancerous lesions, namely, signs of DNA damage, activation of DNA damage checkpoints and also the increase in apoptosis. Due to this, an increase in DSBs occur which forces selection of inactive p53 cells. Interestingly, there is a lot of senescence. Thanks to elegant approaches, a connection between DNA damage and senescence occurs; indeed, oncogene activation of the DNA-damage checkpoints which in turn triggers senescence. Thus senescence is a checkpoint response by oncogenic stress. How do oncogenes induce DNA damage? Thanks to oncogene activation, DNA replication stress occurs and as a consequence, DSBs accumulation. DSB trigger the ATM-Chk2 pathway of apoptosis, and induce apoptosis. In addition, DSBs induces changes in chromatin structure that expose the K79 of histone H3, normally hidden in the nucleosomal core. This further reinforces the checkpoint, by methylation and subsequent recruitment of checkpoint proteins.

Symposium Genetic epidemiology/Whole genome scan

Dr. Ponder (Cambridge, UK) discussed the strategies used for the identification of high-risk susceptibility genes, using breast cancer as a paradigm. Linkage studies have led to the discovery of BRCA1 and BRCA2, and association studies based on candidate genes resulted in the identification of additional genes such as CHECK2, notably thanks to the pooling of data across many studies within the frame of consortia. In recent years, whole genome association (WGA) studies have changed our understanding of breast cancer susceptibility, and have allowed the identification of several new susceptibility variants that were not suspected before. Also in the case of WGA studies, however, replication in large sets of cases and controls is necessary, and this can best be performed within consortia.

Dr. Thomas from US NCI presented the results of the CGEMS study, an effort aimed at conducting GWA studies of prostate and breast cancer, each based on one discovery and two validation phases. Most of the results of the breast cancer WGA have been published, while the second validation of the prostate cancer WGA is still underway. He also discussed how new low-risk variants identified in GWA studies can be used to determine the individual risk of cancer.

Dr. Hung from Toronto presented the results of the GWA study coordinated by the International Agency for Research on Cancer on lung cancer, which has resulted in the identification of the first genetic variant for this neoplasm. This work has been confirmed in two other GWA studies that were reported at the same time, and efforts are currently made to understand whether the genes act through tobacco dependence. Also, in the case of lung cancer, the work is largely conducted within the framework of large international consortia.

Finally, Dr. Weinberg from NIEHS discussed statistical issues in the design, conduct and interpretation of genetic epidemiological studies, in particular those aiming at identifying variants involved in childhood cancer through designs involving genotyping of parents and siblings.

Awards Lecture Antony Dipple Carcinogenesis. This Awards was given to Dr. RA Weinberg who gave his lecture on the mechanism of malignant progression. One of the most interesting aspects of the lecture was the role that epithelial-mesenchymal transition (EMT) had on the generation of cells with stem cell properties. Induction of an EMT on immortalized human mammary epithelial cells results in acquisition of mesenchymal and stem cell markers. These cells had an increased ability of forming mamospheres and stem-like cells isolated from mouse or human mammary glands or mammary carcinomas expressing EMT markers. Increases in human epithelial cells that had undergone EMT and form mamospheres are able to induce more efficiently tumours and soft agar colonies. An analysis of the expression of genes involved in normal stem-cells (ES) in human tumours. They showed that histologically poorly differentiated tumours show preferential overexpression of genes normally enriched in ES cells such as Nanog, Oct4, Sox2, and c-Myc and repression of polycomb-regulated genes. This ES-like signature is preferentially expressed in high-grade-oestrogen negative tumours, those with poor prognostic value. He finally analysed the role of host systemic environment and the contributions of the host to tumour growth. He described how human breast carcinomas instigate the growth of indolent tumour cells, micrometastasis and human tumour surgical specimens located at distant anatomical parts. This process is carried out with incorporation of bone marrow cells to the stroma of the distant indolent tumours. The bone marrow of the host harboring instigating tumours are functionally activated and secrete osteopontin which is necessary for Bone marrow activation and the growth at distance of the non-indolent tumours. These data show an attractive scenario where the growth of non-indolent tumours at distance can be

governed by an endocrine factors, such as osteopontin, released by certain instigating tumours opening the possibility of therapeutic intervention.

Symposium Cancer Cell Metabolism

The increased rate of glucose to lactate conversion displayed by malignant cells even in the presence of oxygen was first described by Otto Warburg in the 20's. In this symposium five speakers discussed tumour progression, molecular adaptation, 'Darwinian' selection and functional imaging in the face of hypoxia, nutrient-deprived and acidic microenvironment.

Jacques Pouyssegur (Nice, France) highlighted the major role played by Hypoxia-Inducible Factor, HIF and by some of the HIF-induced markers in tumour resistance to nutrient-depleted and acidic microenvironment. He showed that the two HIF-induced 'BH3-only'-proteins (BNIP3, BNIP3L), in contrast to the current belief, do not trigger cell death but tumour cell survival by inducing autophagy. Second he showed how tumour cells by expressing two HIF-dependent membrane-bound carbonic anhydrases, CAIX and CAXII, acidify the extracellular milieu, and ensure a more alkaline intracellular pH favoring maintenance of ATP levels and survival in this hostile acidic tumour microenvironment. He provided evidence that interfering with HIF-regulated targets controlling intracellular pH (CAIX, CAXII, MCT-4, NHE1) could have profound impact in tumour regression.

Valeria Fantin (Boston, USA) discussed the common "metabolic" phenotype across cancer types that most likely arises as a consequence of strong selective pressure, conferring emerging neoplastic cells with a significant growth advantage. At the molecular level hypoxia, pseudohypoxia and oncogenes through HIF1-dependent and independent mechanisms are among the drivers of this switch towards a highly glycolytic metabolism. She showed that interfering with pyruvate to lactate conversion or with lactate export by knocking-down LDHA or MCT4 expression respectively renders tumour cells unable to cope with hypoxia and decreases their tumorigenic potential in vivo.

Robert Gillies (Tucson, USA) discussed carcinogenesis as a process of somatic evolution, wherein phenotypes (not genotypes) are selected by ever-changing microenvironmental barriers to proliferation. The first of these barriers is hypoxia, which is established by hyperproliferation of cancer cells away from their blood supplies. This barrier can be overcome by elevated glucose metabolism, which can occur via a number of well-defined mechanisms, including alterations in HIF, c-myc, pAkt, p53, or mitochondrial respiration. Increased glucose metabolism leads to acidosis, which leads to increased invasiveness. This "acid-mediated invasion hypothesis" was tested by chronically neutralizing the released acid via ingestion of bicarbonate, which led to significant reductions in the numbers and sizes of spontaneous metastases in a breast cancer xenograft model."

Eileen White reported that cancer cells require autophagy to eliminate p62-containing protein aggregates and damaged organelles that accumulate in response to metabolic stress. Cancer cells with defects in autophagy (allelic loss of beclin1 or deficiency in atg5) accumulate high levels of p62-containing protein aggregates and damaged mitochondria, which are associated with elevated oxidative stress, activation of the DNA damage response and accelerated tumorigenesis. Moreover, the persistent, high levels of p62 in autophagy-defective cells are responsible for increased oxidative stress and increased tumour growth. Thus autophagy functions as a tumour suppression mechanism by promoting protein quality control and the elimination of p62 in response to stress.

The focus of Zaver Bhujwala's talk (Baltimore, USA) was to present new molecular and functional imaging applications in understanding and targeting cancer metabolism. She presented data to demonstrate the ability of combined MRI and optical imaging to obtain insights into the relationship between hypoxia, vascularization, and choline metabolism. The use of multimodality imaging agents for image-guided combined prodrug enzyme-siRNA targeting of choline kinase in combination with 5-fluorouracil treatment was described. Finally, data on the role of hypoxia in inducing changes at the mRNA level suggestive of a 'breast cancer initiating cell phenotype' were presented.

Symposium: Immune system and cancer

The symposium on Immune System and Cancer highlighted the functional interaction between tumour and its stroma and the possibility of targeting stroma cells for therapeutic purpose.

Laurence Zitvogel (Villejuif, France) showed that cancer cell death elicited by radiotherapy and some chemotherapeutic agents such as anthracyclines is immunogenic. Immunogenic cell death is characterized by the early cell surface exposure of chaperones including calreticulin and/or heat shock proteins, which alert nearby dendritic cells (DC) inducing their uptake of tumour antigens and their maturation. Moreover, the late release of High mobility group box 1 (HMGB1), which acts on toll-like receptor 4 (TLR4), and activation of inflammasome, which allow IL-1b secretion, is also required for optimal immune activation after presentation of antigens from dying tumour cells.

Along the same lines, Han Schreiber (Chicago, USA) presented data showing that local irradiation or a chemotherapeutic drug causes sufficient release of Ag to sensitize stroma cells for destruction by adoptively transferred Ag-specific cytotoxic T lymphocytes (CTLs). Maximum loading of tumour stroma with cancer Ag occurs 2d after treatment, a time window in which T cell transfer should be performed. The above findings may set the stage for developing rational clinical protocols for combining irradiation or chemotherapy with CTL therapy or other forms of immunotherapy.

The cellular component of stroma also has a supporting effect of tumour progression; the most abundant cells in the tumour microenvironment are macrophages and their number often correlates with poor prognosis.

Jeffrey Pollard (Bronx, USA) showed that macrophages are recruited to the tumour invasive front in response to tumour-derived chemotactic factors and because of disrupted basement membrane. Tumour-macrophage loop is established thanks to production of csf-1/M-CSF and of epidermal growth factor (EGF), respectively. Accordingly genetic ablation of macrophages, in mice, impairs tumour progression and metastasis.

Less numerous but not less important is a subset of monocytes that express the angiopoietin receptor Tie2 (TEMs) in driving tumour angiogenesis. Luigi Naldini (Milan, Italy) illustrated the possibility of depleting TEMs selectively with the consequence of halting tumour growth. Moreover thanks to their preferential localization at the site of tumour angiogenesis, it is possible to harness them to release IFN α as a way to deliver, locally, cytokines with immunological and anti-tumour activities.

Plenary lecture: "Inflammation and cancer"

Michael Karin (San Diego, USA) gave a plenary lecture describing recent advances in understanding how inflammation contributes to cancer progression. The idea

that inflammation can promote tumour development is over a hundred years old, but insights into mechanism have been slower to unfold. Much of Dr Karin's work has identified signaling through the NF- κ B family of transcription factors as a key mechanistic link between inflammation and cancer. NF- κ B factors integrate multiple stress stimuli and transduce these into transcriptional programmes that underlie innate and adaptive immune responses associated with inflammatory conditions. NF- κ B can be activated by the kinase IKK α , that is normally found in the cytoplasm. However, IKK α accumulates in the nuclei of cancer cells, both in a mouse model of prostate cancer and in human prostate biopsies. Furthermore, levels of IKK α in nuclei correlate with metastatic progression. A mutation that prevents IKK α activation inhibits metastasis in mice. Chromatin immunoprecipitation revealed that IKK α is present at the promoter of the gene encoding maspin, a potent suppressor of metastasis. Expression of maspin correlates inversely with both nuclear IKK α and with metastatic activity. Indeed, ablation of maspin restores metastatic potential in IKK α mutant tumours. Activation of IKK α in prostate cancer tissue depends on cytokines such as RANK ligand produced by inflammatory cells that are recruited into the growing tumours. These observations lead to a model in which cells expressing inflammatory cytokines infiltrate tumours and trigger nuclear accumulation of activated IKK α which represses expression of the maspin gene and thereby stimulates metastasis.

To explore how inflammatory cells are recruited into tumours, Dr Karin and colleagues screened carcinoma cell lines for their ability to produce soluble factors that activate macrophages and induce cytokine production. They found that the ability of carcinoma cell lines that produce such factors to establish lung and liver metastases is strongly dependent on Toll-like receptor 2 and production of tumour necrosis factor α by cells derived from the host bone marrow. A compelling model was presented in which metastatic progression depends on reciprocal dynamic interactions between the cancer and inflammatory cells, which are recruited into growing tumours to produce pro-metastatic cytokines.

Symposium: Ageing telomerase

In this symposium different aspects of the implication of telomerase in cancer and ageing were shown.

Dr. Gilson (Lyon, France) showed the effects of inhibition of the telomeric protein TRF2 on tumorigenicity. This protein, implicated in promoting the single-strand invasion and T-loop formation, reduced the ability of transformed human fibroblast to form tumour in immunosuppressed mice. There was no sign of growth inhibition, cell cycle defect, apoptosis or senescence after TRF2 dominant negative expression in vitro. These results suggest that TRF2 inhibition leads to a modification of the extracellular environment that prevents neo-angiogenesis.

The second speaker of the session, Dr. Harrington (Edinburgh, UK), showed unpublished data that demonstrated the impact of mTERT heterozygosity particularly upon hematopoietic stem cells. These results demonstrated that upon 10 mTERT +/- generation, telomeres lengths were stabilised relative to previous generations and no phenotypic defects were observed indicating that there is a telomere length adaptation after prolonged exposure to limiting telomerase levels, preserving the HSC function even in successive generation with compromised telomerase.

In his talk Dr. Rudolph (Hannover, Germany) demonstrated that DNA damage checkpoints limit stem cell function in response to telomere dysfunction, suggesting Exonuclease-1 as a possibly target for future therapies, its deletion prolonged lifespan of telomerase dysfunctional mice due to an impairment of ATR recruitment to DNA breaks. In the second part of his talk using a peptidomic approach he

demonstrated that there are biomarkers of human ageing, the levels of these markers are related to ageing disease. These could be predictive markers for cancer risk in aged humans.

Dr. Ligner (Epalinges s/Lausanne, Switzerland) using a single telomerase extension assay demonstrated that telomerase didn't extend every telomere in each cell cycle, in fact short telomeres are elongated preferentially. They had discovered the transcription of the telomeric chromatin into telomeric repeat containing RNA (TERRA). This new described RNA forms part of the telomeric heterochromatin and it seems to play an essential role in telomere structure and regulation.

Presidential Sessions

The two Presidential Sessions each consisted of six presentations, selected from the most relevant ones covering the areas of tumour biology, translational research, oncogenomics and genetic epidemiology. V. Sanz Moreno (London, UK) presented evidence of the GEF-GAP signaling module controlling the movement of metastatic melanoma cells. In a series of melanoma cell lines they were able to show that tumour cells can adopt two different ways of movement: a mesenchymal mode where cells show elongated polarized morphology and an ameboid mode with cells showing a rounded morphology. Both ways of movement were inter-convertibility and reciprocally controlled by rac and rho. Therefore, the expression of the GEF and the GAP determines the way in which different melanoma cells move.

By using transgenic mice ectopically expressing human tenascin-C in pancreatic islets, K Lange (Basel, Switzerland) explored its potential in promoting tumorigenesis. The resulting animals showed normal development of the pancreas but showed enhanced angiogenesis in the pancreatic islets. When crossing these animals with RipTag2 (RT2) mice, the resulting double transgenic developed more frequent and death incidence than RT2 mice and showed several signs of enhanced tumour progression such as appearance of local and distant metastasis.

In order to study the role of breast tumour environment in the function of plasmacytoid dendritic cells (pCD) V Sisirak (Lyon, France), developed different strategies: ex vivo, in vitro and in vivo, using breast cancer cells and a pCD cell line. In vitro studies showed that pCD in the presence of breast tumour supernatants keep their capacity to induce T cell proliferation but direct those cells to produce an immunosuppressive cytokine IL10. TNF α and TGF β seem to be involved in this process suggesting new therapeutic approaches to modulate the tumour suppressive activity of pCD.

A new molecular approach for subclassification of breast carcinomas based of aCGH gene expression, IHC and ploidy was presented by H.G. Russnes (Oslo, Norway). Using tumour tissue from 137 early stage breast cancer patients they designed a CGH classifier based on known genomic alterations characteristic of intrinsic subgroups, and applied a mathematical algorithm on the aCGH data that defines loss or gains in addition to mere complex alterations. Based on this model, they were able to identify luminal, non-luminal, a mixed and an unclassified group of tumours. Different molecular modifications were observed in these groups: luminal type with gains or loss of whole chromosome arms, non-luminal group showed more complex genomic alterations, and this group could be further stratified by HER2 status. Samples in the mixed and the unclassified subgroups were most aneuploid and all the expression subclasses were represented.

A Kong (London, UK) presented a work demonstrating that activation of alternative Her receptors mediates resistance to RGF α tyrosine kinase inhibitors. By using Förster Resonance energy transfer (FRET) which monitors Her2 in situ phosphorylation and classical biochemical analysis they showed that specific TK

inhibitors such as AG1478, Iressa, decreased EGFr and Her3 phosphorylation by inhibition of EGFr/HER 3 dimerization. Consequently HER4 was cleaved and HER4 dimerizes with HER3 leading to persistent Her 2 phosphorylation in the cells that now are resistant to the inhibitors.

B Borgia (Zurich, Switzerland) presented a work on the search for tumour associated antigens localized in newly formed blood vessels in the surrounding stroma of the tumour with the aim to develop an antibody-based therapy relevant in metastatic processes. By using three different syngeneic mouse models and a hepatic metastatic model 9481 different peptides were identified clustering into 1902 proteins. More than 500 were identified exclusively in tumour samples and represent suitable domains for antibody production for therapy purposes.

GL Dalglish (Cambridge, UK) presented a somatic mutational screen in renal cancer. By using a collection of 101 DNA samples from 96 primary cancers and 5 renal cancer cell lines with their matched normal samples, this group was able to identify 300 somatic mutations. The number of mutations varied significantly among individual cancers. Over 200 genes were found to have at least one somatic mutation and in most cases these genes harbored only one or two somatic mutations. This sequencing project allowed that the mutation prevalence and the mutations spectrum of individual tumours to be studied in depth and facilitate comparisons between primary tumours and cancer cell lines.

R Drost (Amsterdam, The Netherlands) presented evidence the role of BRCA1 in breast tumorigenesis, drug response and acquired resistance. By using a conditional mouse model for BRCA1 associated breast cancer that showed that BRCA1 deficient tumours are more sensitive to platinum drugs than to other drugs. BRCA1 mutations lacking exons 5-13 did not develop resistance to cisplatin, suggesting that BRCA1 activity is required for cisplatin resistance. Also preliminary data presented by studying clinical samples of ovarian cancer patients indicated that patients carrying mutations in BRCA1, showed different responses depending on the BRCA1 mutation. This data suggested that a detailed study based on the study of mutations in BRCA1 and cisplatin responses needs to be carried out in order to obtain the best therapeutic results.

L Carvajal Carmona (London, UK) presented a genome-wide association study of tag SNPs in colorectal cancer. This group used two large British case-control cohorts, enriching the discovery phase of the study with cases that had a strong family history of colorectal cancers and with hypernormal control cases. In addition to the three previously identified susceptibility loci two novel association loci 10p14 and 823.3 were found. These loci include POU5F1P1, SMAD7 and EIF3S3.

miRNAs are able to act as tumour suppressors or oncogenes. H. Shen (Nanjing, China) studied SNPs in miRNAs and their surrounding regions and presented evidence demonstrating that rs11614913 and has-mir-1961-2 are significantly associated with survival in non small cell lung carcinoma (NSCLC) and represent an independent prognostic biomarker for NSCLC opening the possibility of using this miRNAs SNPs for therapeutic interventions.

Finally, F Lesueur (Lyon, France) approached the study of ATM breast cancer susceptibility in a pooled analysis of case-control mutations screening data. The objective of the study was to determine the sort of sequence variation in ATM that could confer increased risk to breast cancer. The preliminary data presented indicated that a careful analysis of missense substitutions will have a real utility in case-control mutation screening projects.

Poster presentations

Finally, 657 posters were presented at EACR-20 in three poster sessions. Each day a Prize of 1,000 Euro was awarded by the Pezcoller Foundation for the best poster presentation. The prizes were presented to Inmaculada Ibañez de Cáceres, Spain, Anchit Khanna, Finland and Agnes Csiszar Austria.

We really hope that this Meeting Report provides a view of the scientific excellence of EACR-20 and we invite you to attend to the 21st Biannual Meeting that will take place in Oslo, Norway 26 – 29, June, 2010. Please visit <http://www.eacr.org/> for future details.

Conflict of interest statement

None declared

Acknowledgements

EACR-20 has been a more multidisciplinary meeting of the EACR with numerous sessions, and due to this it has not been possible to cover every aspect of all the excellent sessions and we apologize for the omissions.

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