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First Barcelona Conference on Epigenetics and Cancer

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The Barcelona Conference on Epigenetics and Cancer (BCEC) entitled "Challenges, opportunities and perspectives" took place November 21–22, 2013 in Barcelona. The 2013 BCEC is the first edition of a series of annual conferences jointly organized by five leading research centers in Barcelona. These centers are the Institute of Predictive and Personalized Medicine of Cancer (IMPPC), the Biomedical Campus Bellvitge with its Program of Epigenetics and Cancer Biology (PEBC), the Centre for Genomic Regulation (CRG), the Institute for Biomedical Research (IRB), and the Molecular Biology Institute of Barcelona (IBMB). Manuel Perucho and Marcus Buschbeck from the Institute of Predictive and Personalized Medicine of Cancer put together the scientific program of the first conference broadly covering all aspects of epigenetic research ranging from fundamental molecular research to drug and biomarker development and clinical application. In one and a half days, 23 talks and 50 posters were presented to a completely booked out audience counting 270 participants.

Introduction

In his opening speech, organizer Manuel Perucho highlighted the unique character of these conference series, which are organized as a joint effort of five leading biomedical research institutes located in Barcelona in collaboration with the B-Debate program of the governmental organization BioCat, supported by the Foundation Obra Social La Caixa. The 5-year cycle of conferences will continue with one of the research centers taking the lead each year (**Fig. 1**).

Being the first of the series under the generic title "Challenges, Opportunities and Perspectives," a varied program was arranged to cover cancer epigenetics in its breadth. In these two days, fundamental, translational, and applied aspects of cancer biology and epigenetics were discussed by leading scientists from all around the globe. For more detailed and additional information on this first BCEC please visit: http://www.imppc.org/congress/bcec1/index.html or http://www.bdebate.org/debat/barcelona-conferences-epigenetics-and-cancer.

The Conference served to illustrate how established paradigms are being revisited and often replaced by new ones. A necessarily brief and condensed report of some of the most relevant contents of the talks follows, organized by the overall topics covered in the different sessions of the Conference.

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New Histone Modifications and Novel Insights in Old Ones

While the discovery of acetylation as the first histone modification is celebrating its 50 anniversary, we are still discovering new ones and the end does not seem to be in sight. One example is the heterochromatic trimethylation of histone H3 on lysine 9 (H3K9me3), as discussed by Thomas Jenuwein (Max-Plank-Institute of Immunology and Epigenetics, Freiburg, Germany). He pointed out his counterintuitive and exciting finding that the binding of transcription factors Pax3 and Pax9 was required for H3K9me3 and heterochromatin formation in mouse ES cells.[1] Based on follow up data he suggested a general model in which unorganized transcription factor binding opposed to clustered binding at promoters would initiate heterochromatin formation. Using embryonic stem (ES) cells deficient for several K9-modifying enzymes, Thomas Jenuwein was able to define the criteria for Suv39h-dependent H3K9me3 even further: the DNA sequence has to be repeat-rich, needs to have potential for generating RNA transcripts, and should contain intact transcription factor binding sites. This is exemplified by the Suv39h-dependent repression of intact LINEs and LTRs in the mouse ES cell epigenome.

Several speakers dedicated their time to the description and discussion of new or poorly known histone modifications. Antonis Kirmizis (University of Cyprus, Nicosia, Cyprus), for instance, presented a novel active mark occurring on rDNA (rDNA), the N-terminal acetylation of histone H4 (N-acH4), catalyzed by the yeast enzyme Nat4.[2] This modification directly inhibits the enzyme Hmt1, responsible for the asymmetric dimethylation of arginine 3 (H4R3me2a) linked to repression. Remarkably, under calorie restriction the crosstalk between N-acH4 and H4R3me2a is induced and regulates rDNA silencing, a mechanism that allows cells to respond to environmental stresses such as starvation.

Another rDNA-specific modification identified by Tony Kouzarides (Gurdon Institute Cambridge, Cambridge, UK) by mass spectrometry was glutamine methylation (Qme) on histone H2A. Methylation of Q105 in yeast, a residue highly conserved from yeast to humans, disrupts the binding of FACT chaperone to H2A, prevents FACT from reassembling nucleosomes and leads to enhanced transcription. Remarkably, this modification seems to occur exclusively on the 35S rDNA locus.[3] Tony continued to also disclose the function of arginine citrullination. He showed that citrullination by PADI4 occurred in pluripotent stem cells and that histone H1 was a target. Citrullination of histone H1 on R54 leads to its eviction from chromatin and thus contributes to the open chromatin state intrinsic to pluripotent stem cells.[49]

Sandra Peiró (IMIM, Barcelona, Spain) discussed the role of Snail1 transcription factor in repressing mouse pericentromeric heterochromatin acting through the LOXL2 enzyme.[4] Previously, they could elegantly demonstrate that LOXL2 catalyzes the oxidative deamination of H3K4me3.[5] Oxidative deamination has been suggested to contribute to the transcriptional state required for epithelial to mesenchymal transition.

Challenge: Defining the Epigenetic Regulation of Stem Cells

Developmental studies on the opposing functions of Polycomb (PcG) and Trithorax group of proteins in gene repression and activation, respectively, have largely contributed to the dogma that chromatin modifications form the basis of epigenetic regulation.[6] Kristian Helin (BRIC, University of Copenhagen, Denmark) addressed the open question of whether PcG repressive complexes (PRCs) initiate gene repression or whether their recruitment occurs after silencing. Using mouse ES cell differentiation, they demonstrated that target genes are indeed silenced prior to binding of PRCs. He continued on to demonstrate that, in fact, inhibition of transcription is a prerequisite for PRC recruitment. His evidence suggests that PRC binding to CpG rich regions might indeed be a default state in the absence of transcription. This finding provides important insight to the longstanding question about how PRCs are actually recruited to target genes.

PRCs can be divided in two major types of complexes denominated PRC1 and PRC2. In particular, PRC1 is highly modular and can be composed of different alternative subunits. Luciano di Croce (CRG, Barcelona, Spain) presented data analyzing the functional relevance of the PRC1 modularity. In mouse ES cell differentiation, they reported simultaneously with another group of investigators a switch in the PRC1 composition during differentiation. The Polycomb homolog Cbx7 is predominant in the pluripotent state but replaced by Cbx2, Cbx4, or Cbx8 during differentiation.[7] He further described the non-canonical PRC1 complex containing RYBP protein instead of the canonical Cbx protein.[8] While PRC1-Cbx7 regulated classical developmental target genes, PRC1-RYBP limited the expression of metabolic genes.

Another open question in the Polycomb field is how the transition from a Polycomb-repressed state to full activation occurs, and vice versa. The identification of PHF19 as a protein binding both active mark H3K36me3 as well as the PRC2 complex provided the first insight about how transition from active to repressed state could be achieved.[9] Conversely, Marcus Buschbeck (IMPPC, Barcelona, Spain) showed unpublished data providing a first answer for the opposing transition from the repressed to the active state. During ES cell differentiation, Cbx8-containing PRC1 complexes replace the pluripotency-specific PRC1-Cbx7 complexes on activated genes. This exchange facilitates the initial activation of essential differentiation genes but, after prolonged activation, Cbx8-PRC1 complexes are also evicted. As Cbx8 itself is regulated by differentiation cues, a function for Cbx8-PRC1 as a transition complex is likely to add to the robust execution of the differentiation program. Moving on, Marcus Buschbeck pointed out that a similar contribution to differentiation gene activation had been previously observed for yet another "repressive" factor, the histone variant macroH2A.[10] His yet unpublished rigorous analysis of macroH2A ChIP-sequencing data confirms the previously suggested overlap of macroH2A with the Polycomb mark H3K27me3[11,12] but further pointed out the previously unrecognized existence of another macroH2A-containing type of chromatin. These new regions are characterized by higher local enrichment of macroH2A and lack of H3K27me3, but significant overlap with other marks and binding factors, including CTCF. In light of these findings, the loss of function phenotypes characterized in somatic reprogramming, development, and cancer (summarized by Cantariño et al.[13]) will need to be revisited.

The Trithorax group of proteins was introduced by Jeffrey Dilworth (Ottawa Hospital Research Institute, Ottawa, Canada), who examined the role of Trr-like trithorax complex in muscle specific stem cells. In addition to the methyltransferase MLL4, the complex includes the histone H3K27me3-specific demethylase UTX, another enzymatic active subunit. He could demonstrate that the function of UTX after recruitment by Six4 was essential for muscle regeneration and could not be compensated for by the related enzymes UTY and JMJD3.[14]

A technical but major discussion in the ES cell field concerns how well culture conditions actually reflect the natural states of pluripotent cells in the developing embryo. Henk Stunnenberg (Radboud University, Nijmegen, The Netherlands) presented a tour of force comparative transcriptomic and epigenomic study of mouse ES cells cultured in conventional serum conditions or serum-free 2i medium containing MAPK kinase and Gsk3 inhibitors.[15,16] Major differences can be observed at the transcriptional and epigenomic level. Particular striking is the strong reduction of H3K27me3 and DNA methylation in 2i conditions. Cells can switch between both states and the DNA demethylation observed upon moving cells into 2i medium correlates with repression of DNMTs and is enhanced by Vitamin C, likely through the activation of TET-dioxygenase enzymes. Taken together, the studies from the Stunnenberg lab provide compelling evidence that ES cells cultured in 2i medium represent a naïve ground state resembling best the pre-implantation cells of the inner cell mass of blastocysts, while ES cells grown under conventional conditions are more similar to the post-implantation stage. Another startling observation was the short time required to change the methylation state of the genome by

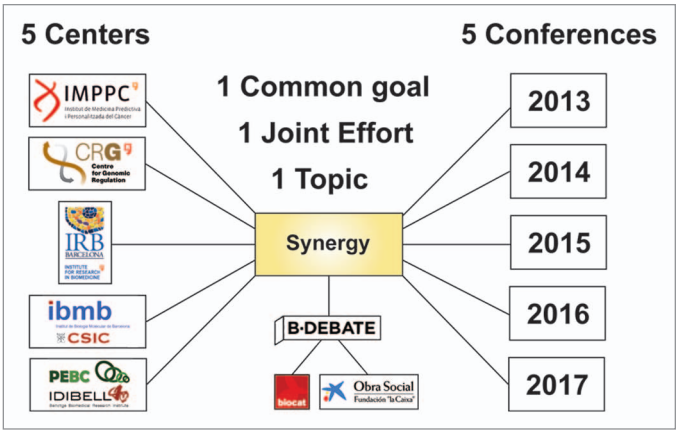

Figure 1. Scheme of the Barcelona Conferences on Epigenetics and Cancer project.

these cells in culture, which implies that DNA demethylation is a very active and widespread process providing bidirectionality to what appeared to be a simplifying ratchet for the process of DNA methylation.

Challenge: Understanding the Three-Dimensional Organization of the Epigenome

The mind-boggling superfast process of fluctuations in chromatin state was continued in the talks dealing with three-dimensional organization of the epigenome. Levering chromosome capture approaches with next generation sequencing such as 4C-seq, 5C, or Hi-C techniques has lead us to an entirely new level of understanding of the three-dimensional organization of chromatin. Bing Ren (Ludwig Institute, University of California, San Diego, USA) used these techniques to study the organization of the genome on the level of topological domains (TADs) and their relative position to each other. His modeling of an entire mouse chromosome and the integration of ChIP-seq data revealed a separate clustering of TADs with and without H3K9me3 in different compartments of the chromosome.[17] Another thought-provoking issue is the widespread phenomenon of allelic bias in gene expression. According to Ren's estimations, around 20% of all genes manifest allelic specific expression. This has implications for the understanding of the consequences of loss of heterozygosity (LOH) in aneuploid cancer cells. The sequence basis of Hi-C data can indeed inform on haplotypes.[18]

Marc Martí-Renom (CNAG, Barcelona, Spain) discussed how external stimuli impact on TADs using progesterone treatment of breast cancer cells as a model system. In depth analysis revealed that TADs are homogenous at the epigenomic marking level. TADs homogenously responded to hormone treatment in that all embedded responsive genes were regulated in the same manner. Using 3D models, he and his collaborators Guillaume Filion and Miguel Beato could demonstrate that activated TADs expanded, thereby increasing accessibility, whereas repressed TADs contracted their chromatin structure resulting in lower accessibility.

Drosophila insulator binding proteins (IBPs) define the borders between euchromatin and heterochromatic domains, often coinciding with borders between TADs. IBPs further bind nucleosome-free regions. Olivier Cuvier (LBME-CNRS, Toulouse, France) discussed unpublished data showing that IBPs bind the H3K36me2-specific histone methylase dMes4. This interaction was required for the recruitment of the transcriptional co-activator Dref, the opening of chromatin, nucleosome positioning, and H3K36me3-prone splicing. Furthermore, Olivier reported a novel procedure to detect specific long-range interactions at high resolution, based on the detection of ChIP-indirect peaks, as confirmed by aggregating Hi-C data on such peaks.[19]

Raffaella Santoro (University of Zurich, Zurich, Switzerland) highlighted the nucleolus and its rDNA as an important center of nuclear architecture and core to epigenetic regulation. During differentiation of ES cells a portion of rDNA copies adopts a heterochromatic state, which, extending on her previous work,[20] she found is dependent on the recruitment of DNMTs and HMTs by non-coding pRNA-bound Tip5. Alterations of rDNA heterochromatin affected also heterochromatin from other genomic regions, possibly through direct contact on the nucleolar surface. Overexpression of functional pRNA in ES cells induces heterochromatinization, while not affecting ribosomal biosynthesis. Excitingly, pRNA-overexpressing cells had lost the capacity to form teratomas, which could be a direct consequence of changes in nuclear organization.

Amos Tanay (Weizmann Institute of Science, Rehovot, Israel) started his talk by remarking that new techniques booming in single-cell genomics allow reviewing fundamental questions in epigenetics. Some of these questions are how many cell types exist, what is the diversity within a given cell population and how can we distinguish a cellular state of activation from cell type. Even though in a premature phase, he showed that performing single-cell transcriptome helps redefining cell types in analogy to a cell sorter using hundreds of markers. Amos further aims to exploit these techniques to understand how cell states are being memorized and which is the mechanism underlying it. Although not a single-cell technique per se but an approximation, they have further expanded single clones to address the noise and memory of DNA methylation patterns in pluripotent and differentiated cells comparing ES cells and fibroblasts, respectively.[21] The conclusion of their elegant study is that ES cells have no clonal memory while every fibroblast clone memorized a different type of noisy pattern during its clonal evolution. He suggested that no memory in an intrinsic noisy biological system could be the way by which ES cells maintain and propagate their full potential, avoiding the transmission of stochastic modification patterns.

Opportunities: Taking Advantage of Cancer-Associated Alterations in DNA Methylation

Focusing on cancer epigenetics, Andrew Feinberg (Johns Hopkins University, Baltimore, USA) discussed both differentially methylated regions (DMRs) as well as large-scale nuclear blocks involved in nuclear plasticity. He pointed out that most of aberrant cancer methylation occurred at the shores of CpG

Figure 2. Posters of the BCEC editions 2013 and 2014.

islands and that cancer DMRs resembled those DMRs between tissues. Using both the array technology CHARM as well as whole-genome bisulfite sequencing, Andrew was able to describe large hypomethylated "blocks" in cancer showing hypervariable expression.[22] He suggested that these blocks may be universal defining features of human cancers and changes in CpG islands and shores may be enriched within these regions.

Hypermethylation of CpG islands and hypomethylation of gene regions thus represent a relative increase or decrease in methylation levels that is consequence of the randomization of previous methylation status, with CpG islands normally demethylated and gene regions usually methylated. Blocks have uniform histone modification patterns, overlap with lamina associated domains, increase during stem cell differentiation and are involved in the epithelial-to-mesenchymal transition.[23,24] In his concluding remarks, Andrew stated that loss of epigenetic stability leads to hypervariable methylation, causing tumor heterogeneity, which perhaps can predict cancer risk.[25] As such, we should look at cancer as heterogeneous cell populations with dysregulated epigenomes that allow cellular growth advantage at the expense of the host.[26]

Manuel Perucho (IMPPC, Barcelona, Spain) examined DNA methylation in colorectal cancer (CRC). First, he pointed out that in several instances, like in the pathway for CRC of the microsatellite mutator phenotype, epigenetic alterations in DNA hypermethylation occur first and influence the generation of genetic alterations in CRC. However, once this transformation happens, the genetic alterations supersede the epigenetic alterations driving the cancer phenotype.[27] On the other hand, he analyzed DNA hypomethylation events and proposed a "wear and tear" stochastic model for neoplastic transformation wherein accumulation of errors in methylation replication occurs in the colon crypt stem cells during aging.[28] He presented two examples of DNA demethylation in CRC, which didn't fit in the "wear and tear" model. The first one, already published,[29] showed that LINE-1 repetitive sequence hypomethylation in normal mucosa can be used as an epigenetic predictive biomarker for developing metachronous tumors. The stronger association of demethylation in normal mucosa with multiple CRC risk from relatively younger patients also suggests that endogenous genetic factors may underlie the increased risk to develop multiple tumors. The second, unpublished, example studied DNA hypomethylation in SST1 pericentromeric repeats, present in a 20% of CRC. Severe demethylation in a fraction of cancers occurred in a non-age-dependent manner and correlated with genomic damage, especially in wild type p53 tumors.

Angela Risch (DKFZ, Heidelberg, Germany) talked about lung cancer susceptibility loci identified by genome-wide association studies. In a recently published work, they characterized the *CHRNB4* gene as hypomethylated and associated with increased expression of CHRNB4 in lung cancer. *CHRNB4* is strongly associated with genetic lung cancer susceptibility variants and displays tumorigenic potential.[30] They further used genome-wide association data to identify copy number gain regions associated with lung cancer risk, one of which included a miRNA later shown to be hypomethylated and upregulated in non-small cell lung cancer.

While we primarily consider CpG methylation, Angelika Merkel (Group of Simon Heath, CNAG, Barcelona, Spain) pointed out the presence of non-CpG methylation in B-cells. As member of the BLUEPRINT consortium, she is mining whole genome bisulfite sequencing and could identify an overall reduction of non-CpG methylation during B-cell differentiation. Retained methylated non-CpG sites in differentiated B-cells correlated negatively with lamina-associated domains. Interestingly, the predominant motif was CAC and not CAG, the one reported in stem cells. Study of the dynamics between non-CpG and CpG methylation suggested that non-CpG methylation is not a side effect of de novo CpG methylation. The functional relevance of non-CpG methylation remains to be assessed but the phenomenon seems to be more widespread than previously thought and perhaps the tip of an iceberg and another impending change of paradigm.

It is clear that changes in DNA methylation patterns are a hallmark of cancer that can be exploited for biomarker purposes. Manel Esteller (PEBC, IDIBELL, L'Hospitalet de Llobregat, Spain) provided several examples, including: 1) methylation of the repair gene *MGMT* is the best predictor for a positive response to carbazine treatment. Noteworthy, *MGMT* has been one of the first aberrantly methylated genes identified in cancer and has since been reconfirmed as one of the most relevant in several genome-wide studies; 2) DNA methylation patterns and cross comparison to data sets collected from 1600 tissue and disease samples allows the identification of the tissue of origin in metastasis of unknown primary.[31] A kit to commercialize this assay is in preparation.

Challenge: Exploiting Epigenetic Knowledge for Therapeutic Approaches

Marjorie Brand (Ottawa Hospital Research Institute, Ottawa, Canada) described that TAL1 is not only a master regulator of hematopoiesis but also causative for T-cell acute lymphoblastic leukemia (T-ALL).[32] This dual role of TAL1 in normal hematopoiesis and T-ALL is based on its capacity to activate and repress transcription and to associate with both co-repressors and co-activators. One of these proteins is UTX, the previously mentioned H3K27me3-specific demethylase. A quarter of T-ALL patients have mutations in Ezh2, the enzymatic subunit of PRC2 responsible for the deposition of the H3K27me3. Brand demonstrated that TAL1 recruits UTX, which sustains gene activation by removal of the H3K27me3 mark. Her results showing that loss of UTX both diminished cell growth and increased apoptosis identified UTX as a bona fide drug target in T-ALL and warrant further development of therapeutic approaches that could be beneficial for a patient cohort overexpressing TAL-1.

The BET family of acetyl readers are another set of validated drug targets, as explained by Tony Kouzarides. Small compound inhibitors, such as I-BET, inhibit the recruitment of BET acetyl-readers to chromatin. In this way, the action of aberrant transcription complexes, including those formed by MLL-fusion proteins, is disrupted. Several BET inhibitors have been developed and their efficacy has been confirmed in MLL-fusion positive leukemia[33] but also acute myeloid leukemia[34] and myeloproliferative neoplasms.[35] Currently I-BETs are being tested in clinical trials.

Manfred Jung (Universität Freiburg, Freiburg, Germany) provided a view from the medicinal chemistry side. Using the parasite *Schistosoma mansoni* as target, he demonstrated how small compound inhibitors can be generated against epigenetically relevant enzymes. He decided to focus on the histone deacetylase SmHDAC8, as its loss of function is lethal for the parasite. Exploiting differences in the structure of human and parasite HDACs, the Jung lab was able to generate drugs that are highly effective on SmHDAC8 but have decreased effects on key enzymes of the human host.[36] These drugs are now further optimized for potency and selectivity.

Carlo M Croce (Ohio State University, Columbus, USA) mused on another change of paradigm in the field showing the universal involvement of microRNAs in gene expression regulation and, consequently, in cancer pathogenesis, and described his own contribution when he started to look almost three decades ago, at a region with recurrent chromosomal alterations in the most common leukemia, chronic lymphocytic leukemia (CLL). This was a perplexing gene-free region but, eventually—fulfilling the Sherlock Holmes precept that when you have eliminated the impossible, whatever remains, however improbable, must be the truth—, they discovered that the culprits were two small non-coding microRNAs (miRs), specifically, miR15 and miR16. These miRs contribute to the pathogenesis of CLL and, based on miR arrays, CLL can be classified between indolent or aggressive type. Some of them have shown to work as prognosis markers in CLL.[37] On a cancer context, miRs can work as oncogenes or tumor suppressor genes depending on the cellular context and a simple dysregulation of one miR can lead to cancer in a fast way.[38] A comprehensive signature study in 25 000 tumors (6 different solid tumors) pinpointed some miRs dysregulated in many tumors. As an example, miR21 was found dysregulated in all 6 types of tumors. Interestingly, he remarked that miRs are always targetable, the main issue is to target the right one in a context-dependent manner.

In addition, globally deregulated miR biogenesis can contribute to cancer, as pointed out by Manel Esteller. Inactivating mutations in the *DICER1* gene promoted metastasis in some colon cancer cases.[39] In another study centered on colorectal cancer, his team was able to show that intronic RNAs bind to EZH2, the catalytic component of PRC2, and guide it to repress the locus of origin.[40] The role of epigenetic regulators such as PRCs is not to establish but rather to maintain cell identity by providing stability

to transcriptional programs. In a cancer context, changes in the activity of chromatin regulators, by excess or defect, can lead to misbalanced levels of chromatin modifications, which can be targeted for anti-cancer therapy. Up to this day, four "epigenetic" drugs interfering with chromatin modifying enzymes have been approved by the FDA for cancer treatment but many more are in clinical trials (reviewed in[41]). Approved drugs include the two DNMT inhibitors 5-azacytidine and 5-aza-2-deoxycytidine (traded under the names vidaza and decitabine, respectively). Manel further outlined an exciting example of drug repositioning in which the antibiotic mythramycin was found to have a growth inhibitory effect in lung cancers carrying amplifications of gene encoding the histone methyltransferase SETDB1.[42]

Perspective: Maturing iPS Technology for Regenerative Medicine

Rudolf Jaenisch (Whitehead Institute, MIT, Boston, USA) gave an exciting keynote lecture on the issues that need to be solved to take full advantage of induced pluripotent stem cells (iPS) and disease modeling. The ultimate goal of iPS cell research is to enable us to create genetically defined models for complex diseases and generate unlimited numbers of cells for "customized" tissue repair. Disease modeling using iPS technology has four main issues that need to be solved: the process of iPS generation itself, the ability of controlled genetic editing, defined differentiation protocols to generate the required cell type and tissue transplantation. Major advances have been made on the first two issues. Genetically intact iPS cells of high quality can nowadays be generated using non-integrative tools for the transfer of the Yamanaka factors, such as mRNA transfection or non-integrative viruses.

The introduction of gene editing techniques has opened the door to efficient and controlled generation of cells carrying a specific disease mutation and isogenic controls derived from the same donor. In particular, the possibilities offered by the RNA-based CRISPR/Cas9 system[43] outshine earlier techniques based on Zinc fingers or TAL effectors in terms of efficiency and specificity. It is getting increasingly easy to introduce mutations, deletions, and basically any type of genetic alteration in a controlled manner. To give an example, Rudolf highlighted the simultaneous introduction of five genetic alterations with sufficient efficiency.[44] In addition, unsuccessful differentiation is also a main problem as we often get immature cells; clear examples are hematopoietic stem cells or endoderm (β cells or liver cells). At least some differentiation protocols have reached maturity. These include neuronal differentiation and, as a consequence, RETT syndrome, a disorder of the autism spectrum caused by mutation of methyl CpG binding protein MeCP2, which has been modeled using genetic engineered cells. What came as a surprise to the chromatin field was that, in neurons, MeCP2 seems to act as global activator of gene transcription.[45] In many cell types, including neuronal precursor cells, MeCP2 has well-described repressive functions and, in particular, the early work by Adrian Bird delivered the paradigm for DNA methylation mediated repression.[46] Finally, considering transplantation it is key to select the right cell for transplantation (stem cell, committed, differentiated) and the correct delivery system for functional integration into host tissues. Rudolph concluded that the field of regenerative medicine has entered an amazing phase where an expanding number of tools provide enormous potential. Customized tissue repair may not be imminent, but it might arrive sooner than we imagine and the limit to what will be and what will never be possible is hard to delimit.

Conclusion

We are living in exciting times. This is the main conclusion reached by organizers, speakers, and participants. New fundamental principles are still being discovered and old paradigms are revisited and often replaced by new ones on a regular basis. Novel techniques have provided us a recent quantum leap in our exploratory possibilities. The C-technology, massive parallel sequencing and sub-diffraction microscopy allow us to obtain unprecedented insight in the three dimensional organization of the genome. Novel genome editing techniques, as highlighted by Rudolf Jaenisch, allow us to generate cellular models with unparalleled precision. And the rise of single-cell techniques starts to allow us to overcome the limitations of averaging techniques and to appreciate population diversity. It has further opened the door to assess the stochastic elements of chromatin and epigenetic regulation.

Never before has it been so clear that epigenetic research is highly relevant. We are beginning to get rational clues to explain the link between aging and cancer by the irremediable accumulation of somatic epigenetic errors, particularly in the form of DNA hyper-and hypo-methylation, by the long mitotic history of stem cells in those fortunate of us who reach old age, and highlighting the importance of these epigenetic errors as begetting subsequent "driver" oncogenic alterations. Last but not least, the massive cancer genome sequencing efforts conducted by the cancer genetics community have unequivocally identified chromatin regulators as important driver genes of carcinogenesis.[47] First, "epigenetic" drugs interfering with chromatin regulators have reached clinical application, many more are in clinical trials, and even more targets are currently validated in labs around the globe. Epigenetics has reached mainstream oncology and will, without doubt, further expand its place. We are greatly looking forward to discussing further developments in the coming editions of the Barcelona Conferences on Epigenetics and Cancer.

Outlook

The next edition of the Barcelona Conference on Epigenetics and Cancer will be held October 2–4, 2014 (**Fig. 2**). Organized under the leadership of Alejandro Vaquero, Esteban Ballestar, and Manel Esteller from the PEBC, IDIBELL (L'Hospitalet de Llobregat, Spain) and Juan Ausió from Victoria University (Victoria, Canada), the conference program will commemorate the identification of the first histone modifications 50 years ago by Allfrey, Faulkner, and Mirsky.[48] These seminal findings will

be put into the context of the state-of-the-art research in cancer epigenetics.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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