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THE GLOBAL STEM CELL EVENT

POSTER ABSTRACT BOOK

ENVISION & ENGAGE

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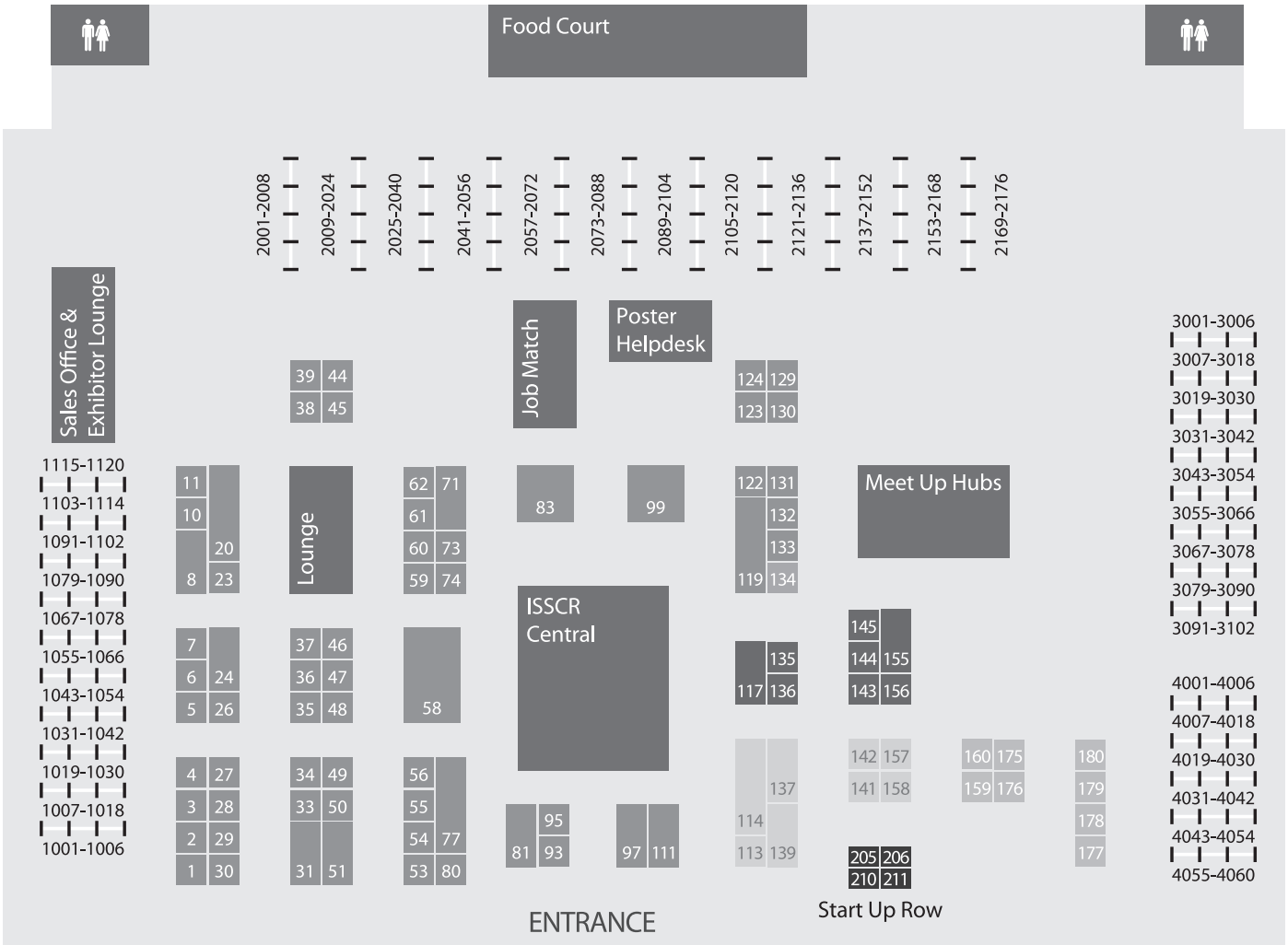

Technology and Suppliers Pavilion

Academic Pavilion

Therapeutic and Commercialization Pavilion

Start Up Row

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TABLE OF CONTENTS

WEDNESDAY, 20 JUNE, 2018

POSTER SESSION I-ODD 18:30 – 19:30

LATE BREAKING ABSTRACTS POSTER SESSION I-ODD 18:30 – 19:30

POSTER SESSION I-EVEN 19:30 – 20:30

LATE BREAKING ABSTRACTS POSTER SESSION I-EVEN 19:30 – 20:30

THURSDAY, 21 JUNE, 2018

POSTER SESSION II-ODD 18:00 – 19:00

TABLE OF CONTENTS

TABLE OF CONTENTS

T-2062

DEVELOPMENT OF AN HYDROXYMETHYLGLUTHARYL-COENZYME A REDUCTASE (HMGCR) OVEREXPRESSION SYSTEM FOR THE STUDY OF REPROGRAMMING TO STEM-LIKE STATES IN HUMAN BREAST CANCER

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The rate-limiting enzyme in the mevalonic acid (MVA) pathway, hydroxymethylglutharyl-coenzyme A reductase (HMGCR), is deregulated in tumors, increasing the synthesis de novo of cholesterol, critical for cell survival and proliferation. However, the role of HMGCR in the induction and maintenance of stemness in both transformed and non-transformed cells is still unclear. Therefore, we set out to induce an HMGCR-on phenotype in the breast cancer (BC)-derived cell line MCF-7, to evaluate whether this phenotype facilitates the acquisition of stem-like traits in BC. With this purpose, we developed an HMGCR overexpression model taking advantage of a CRISPR-on system (dCas9-VP160), which includes expression plasmids for guide RNAs (pSPgRNAs) and a plasmid carrying the sequence coding for the dCAS9. Five guide RNAs (gRNAs) targeted to the promoter of the human HMGCR gene were designed with the informatics tools Genome Engineering Toolbox from the Zhang Lab (MIT, Cambridge, MA) and CRISPR-ERA. The gRNAs and the dCAS9 were then co-transfected into MCF-7 cells, and the levels of total HMGCR was assessed by qRT-PCR at 2 days post-transfection. The CRISPR-dCAS9 system increased HMGCR total levels in MCF-7 cells (MCF-7/CR) a x=2,46; sd=0,4-fold ($p<0,05$) when compared to transfection controls (MCF-7/TC). Interestingly, changes in HMGCR levels in MCF-7/CR and MCF-7/TC cells correlated with corresponding changes in the frequency of stem cells (R2=1), as measured by mammosphere formation assay by limiting dilution and statistical analysis with a specialized software (http://bioinf.wehi.edu.au/software/elda). Additionally, the pluripotency markers Oct4 and Nanog were increased in MCF-7/CR cells (x=1,41; sd=0,36 and x=2,87; sd=0,66 -fold, respectively) at the transcriptional level. To further study the relationship between HMGCR and pluripotency, HMGCR expression was assessed by qRT-PCR in the embryonic stem cell line hES9, and found to be increased to levels comparable to those observed in the HMGCRon models (2,25-fold vs. MCF-7 cells). These data suggest that cellular models expressing a HMGCR-on phenotype may offer useful tools for the study of metabolic phenotypes prone to acquire stem-like traits.

CHROMATIN AND EPIGENETICS

T-2064

CHROMATIN 3D STRUCTURE REMAINS UNCHANGED IN ANEUPLOID EMBRYONIC STEM CELLS

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Chromosome mis-segregation during mitosis or meiosis generates aneuploid daughter cells. It brings costs and benefits to cell fitness under different conditions, and elicits both common cellular responses and context-specific phenotypes of aneuploid cells. However, how the extra chromosomes influence the overall fitness of an aneuploid cell population growing in a particular environment is not fully understood. In recent years,