FIRST SESSION: CELL COMPETITION IN DEVELOPMENT

Genetic analysis of Minute cell competition

Nicholas Baker
Albert Einstein College. New York. USA.

Ribosomal protein genes are usually haploinsufficient and their mutations lead to dominant growth defects. Heterozygous cells are out-competed by wild type cells. We isolated mutations that prevent the loss of Minute cells from mosaic eye and wing imaginal discs. These mutations suggest that regulatory pathways monitor ribosome assembly and are responsible for features of the Minute phenotype.

One mutation corresponds to a mis-sense allele of the *rps12* gene. RpS12 is an essential ribosomal protein but is not Minute ie its mutations are recessive and *rpS12*/+ cells are not outcompeted. The new allele defines a role for RpS12 in cell competition that is separable from its essential ribosomal function. Our findings indicate that, opposite to most ribosomal proteins, wild type RpS12 promotes cell competition. RpS12 defines the competitiveness of Minute cells, cells with higher *rpS12* gene dose being out-competed. The new mutant allele prevents cell competition, without affecting the slow growth of Minute animals. We propose that RpS12 behaves as a sensor of ribosome assembly that is required to trigger cell competition when mutations affect other ribosomal proteins.

Live analysis of Myc-regulated spontaneous competition in embryonic pluripotent cells

Miguel Torres
Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC). Madrid. Spain.

Multi-cellular organisms have evolved tissue homeostasis mechanisms to ensure life-long fitness of their organs and systems. Among these mechanisms, those that regulate the elimination of unwanted cells are fundamental for tissue development and

homeostasis. An important mechanism in this context is the phenomenon of cell competition, by which cells of growing organs are able to compare their fitness with neighbours, and the less-fit but otherwise viable cells are eliminated when confronted with a fitter cell population. Cell competition is executed through the apoptotic elimination of the less-fit population by cell non-autonomous mechanisms. This process is potentially important for the long-term maintenance of tissue performance, as it might provide a mechanism for elimination of suboptimal cells from stem-cell niches and progenitor-cell pools.

Using a genetic mosaic approach in the mouse, we identified Myc-mediated endogenous cell competition in the mouse epiblast as a mechanism for the selection of cells with higher anabolic activity for contribution to the developing embryo. Extension of these studies to cardiac development and cardiac adult homeostasis indicates that the epicardial precursors and cardiomyocytes themselves are sensitive to Myc-induced competition. Stimulation of cell competition in the developing and adult myocardium results in the phenotypically silent replacement of wild type cardiomyocytes by the Myc-overexpressing cardiomyocytes. These results indicate that during cardiogenesis different pools of cardiac progenitors compete for occupying the cardiomyocyte lineage niche without affecting the cardiac morphogenic plan. Currently we are exploring the dynamics and mechanisms of cell competition by time-lapse analysis of an Embryonic Stem cell competition model.

In vivo analysis of spontaneous competitive apoptosis in Drosophila epidermis

Yuka Hayashi[1)], Yuhei Kawamoto[1)], Ayako Isomura[1)], Erina Kuranaga[1)2)]
[1)] Laboratory for Histogenetic Dynamics, RIKEN CDB. Kobe. Japan.
[2)] Laboratory for Histogenetic Dynamics, Graduate School of Life Sciences, Tohoku University. Japan.

Cell competition is a one of cell-cell communication in which cells with low fitness “loser” are eliminated and replace by high fitness cells “winner”. Cell competition has been hypothesized to contribute to homeostasis of organ growth and tumor suppressor by keeping the cell number, however, the molecular mechanism underlying the coordinated regulation between proliferation and apoptosis remains elusive. During the replacement of abdominal epithelium in *Drosophila*, the adult epidermal precursor cells (histoblasts) trigger non-autonomous apoptosis of neighboring larval epidermal cells (LECs) at the histoblast/LECs boundary, and this process maintains the epithelial integrity of the

abdominal region during metamorphosis. We have analyzed the competitive apoptosis in LECs induced by neighboring histoblasts in order to find the mechanistic insight of physiological cell competition. To elucidate the molecules that trigger competitive apoptosis in LECs, we have performed genetic screens using RNAi strains. In our screening, genes predicted to encode a transmembrane or secreted protein were selected by algorithm. As a result, 58 RNAi strains that showed morphological defect of adult epidermis were identified. PDGF- and VEGF-related factor 1(Pvf1) was identified as a candidate gene that functions in histoblasts to induce LECs apoptosis. We herein present the result of RNAi screening and discuss the involvement of Pvf1 with competitive apoptosis in physiological context.

Metabolic control of tissue repair and homeostasis in Drosophila

Masayuki Miura
Tokio University. Japan.

Methionine is one of the essential amino acids and a precursor of S-adenosylmethionine (SAM). SAM is required for methylation of many substrates, such as proteins including histones, nucleic acids, metabolites and lipids. SAM provides a methyl group for substrate and becomes SAH. SAH is then metabolized into homocysteine which is either regenerated into methionine, or converted into cystathionine through the transsulfuration pathway. SAM also provides an aminopropyl group to synthesize polyamines. Using LC-MS/MS analysis, we demonstrated a decrease in Met and SAM, and an increase in SAH in the fat body during the early stage of tissue repair/regeneration after *Drosophila* wing imaginal disc ablation. By combining a temperature-sensitive form of the diphtheria toxin A domain (DtA^{ts})-induced wing disc ablation with the Q system and a fat body-specific Gal4 driver, we manipulated methionine metabolism enzymes specifically in the fat body. Both knockdown and overexpression of the glycine N-methyltransferase (Gnmt), a predominant consumer of SAM for converting glycine into sarcosine in fat body, or SAM synthase (sams) in the fat body impaired the repair of wing discs. These genetic manipulations had no effects on wing development under non-disc ablated flies. These results indicate that the appropriate regulation of methionine metabolism in the fat body is necessary for disc repair, though it is still unclear how both up- and down-regulation of *gnmt* or *sams* impairs disc repair.

The *Drosophila* adult gut homeostasis is maintained by intestinal stem cells (ISC). Under starved condition, ISC proliferation is severely impaired. Genetic analysis revealed that the SAM reduction in ISC prevented proliferation of the ISC. We also found that the SAM depletion induced *upd3* up-regulation in the enterocytes, nutrient absorption cells in the gut, and that the up-regulated Upd3 acts as a survival factor for ISC during starvation. Therefore, SAM governs the gut homeostasis during nutrient starvation in both cell autonomous and non-cell autonomous manner.

Epithelial cell turnover corrects developmental distortion: a possible role of cell competition in morphogenetic robustness

Tatsushi Igaki
Kyoto University. Japan.

Highly reproducible animal development is achieved by robust, time-dependent morphogenesis through cell-cell communications. To study the mechanisms underlying morphogenetic robustness, we analyzed the developmental process of *Drosophila Minute*/+ mutants, a series of mutants heterozygous for a ribosomal protein gene. It has been well known that *Minute*/+ animals show significant developmental delay during the larval period but finally develop into essentially normal flies. Intriguingly, we found that both cell death and compensatory cell proliferation are dramatically increased in the wing imaginal epithelium of *Minute*/+ larvae. Blocking this massive 'cell-turnover' resulted in significant morphological defects, indicating the essential role of cell-turnover in normal wing morphogenesis of *Minute*/+ animals. Interestingly, a forced reduction in Wg signaling gradient in the *Minute*/+ wing pouch by either downregulating or uniformly overexpressing Wg abolished the dramatically enhanced cell-turnover, suggesting the role of Wg-dependent cell competition in this process. The molecular mechanism by which *Minute*/+ wing disc causes massive cell-turnover will be discussed.

How does cell competition contribute to animal development?

Laura A Johnston, Cora Bergantiños, Lale Alpar, Albana Kodra, Marcello Ziosi, Kiki Kanakousaki, Christina Cary and Tim Crawley
Department of Genetics and Development, College of Physicians and Surgeons, Department of Biological Sciences, Columbia University, New York, NY, USA.

Cells in developing tissues and organs behave as social communities and use conserved mechanisms that allow them to cooperate or compete with their neighbors. These mechanisms allow cells to rapidly sense and respond to growth changes in their immediate environment, help to promote appropriate size control and ensure optimal organ fitness. One such mechanism, called cell competition, monitors cellular fitness so that potentially unfit cells are prevented from contributing to the tissue. Cell competition induced by local differences in either Myc or Rp gene dose utilizes specific components of the evolutionarily ancient innate immune system, including Toll-related receptors (TRRs) and the cytokine Spätzle, and lead to NFκ-B-dependent apoptosis in the loser cells, indicating a functional repurposing of components of this signaling module in the surveillance of cell fitness during

development. Different "loser" cells require different TRRs and NFκ-B factors and activate distinct pro-death genes, consistent with a response that is stipulated by the competitive context. I will report on recent work that extend these findings, and discuss our efforts to explore endogenous roles of fitness sensing in animal development.

Supported by the NIH, the NCI, the Lymphoma and Leukemia Society, and EMBO.

SECOND SESSION: MECHANISMS OF CELL COMPETITION

Three types of cell competition: resources, direct fitness comparison and mechanical

Eduardo Moreno
Champalimaud Centre. Lisbon. Portugal.

During my talk I will argue that in Drosophila there are three different and non-exclusive modes of competition: competition for extracellular survival factors (Moreno et al., 2002), competition through fitness fingerprints (Rhiner et al., 2010; Merino et al., 2015), and competition through mechanical stress (Levayer et al. Curr. Bio., 2016). I will discuss the differences and similarities between the three types and their role in ageing, development and cancer.

Cell Competition and Warburg Effect

Yasuyuki Fujita
Hokkaido University. Sapporo. Japan.

At the initial step of carcinogenesis, transformation occurs in a single cell within an epithelial sheet, and the emerging transformed cells grow while being surrounded by normal epithelial cells. However, it was not clear what happens at the boundary between normal and transformed cells. Using newly established cell culture and mouse model systems, we have shown that various phenomena can occur at the interface between normal and transformed epithelial cells. For example, when Ras- or Src-transformed cells are surrounded by normal epithelial cells, various signaling pathways are activated in the transformed cells and they are often eliminated from the apical surface of the epithelial monolayer. These phenomena are not observed when transformed cells alone are

present, suggesting that the presence of surrounding normal cells affects the signaling pathways and fate of transformed cells. Furthermore, we have demonstrated that normal epithelial cells can recognize and actively eliminate neighboring transformed cells and named this process EDAC (Epithelial Defense Against Cancer).

Recently, we have found that mitochondrial activity is substantially decreased in RasV12-transformed cells when they are surrounded by normal epithelial cells. Increased expression of PDK4 is responsible for the down-regulation of mitochondrial activity. Addition of DCA (Dichloroacetate), an inhibitor of PDKs, significantly suppresses the apical extrusion of RasV12-transformed cells, suggesting that PDK-mediated mitochondrial down-regulation plays a positive role in the elimination of transformed cells. Furthermore, expression of LDH is enhanced in RasV12-transformed cells surrounded by normal cells, and suppression of LDH activity leads to formation of basal protrusions. These data suggest that the Warburg effect-like phenotype can occur at the initial stage of carcinogenesis, which plays a tumor-suppressive role by promoting elimination of transformed cells from epithelial tissues. By further developing this new research field, we would create a novel type of cancer treatment: eradication of transformed cells by enhancing a defensive force of neighbouring normal epithelial cells.

How the JAK/STAT pathway controls competitive interactions between cells

Erika Bach
New York University. USA.

During development, local competition between cells impacts which ones contribute to the adult. In *Drosophila* imaginal epithelia, cell competition is induced in adjacent cell populations that differ in metabolic rates. Cells with higher metabolic rates (called winners) cause the apoptotic death of cells with lower rates (called losers). Several conserved proteins - including the transcription factors STAT and Myc - are key regulators of cell competition. Super-competitors are a distinct type of a winners that can out-compete wild-type cells located several cell diameters away. However, the mechanisms used by these cells to eliminate their neighbors are not well understood. We previously demonstrated that STAT super-competitors do not upregulate Myc or its targets, suggesting that these factors function independently in this process. In order to study the transcriptome in STAT super-competitors, we performed next-generation sequencing on RNA from FACS-purified STAT winners isolated from imaginal discs. We compared these data to RNA-seq data from purified wild-type wing disc cells and from Myc super-competitors. Through these analyses and subsequent validation and functional assays, we have identified *Drosophila* Insulin-like

protein 8 (Dilp8) as a factor secreted by STAT super-competitors that controls cell competition. Results from these studies will be presented at the symposium.

---

mTOR regulation of cell fitness during early mammalian development

Sarah Bowling[1&2], Aida di Gregorio[1], Margarida Sancho[1], Jesus Gil[2] and Tristan Rodriguez[1]

[1]BHF Centre for Research excellence, National Heart and Lung Institute, Imperial College London, Hammersmith Hospital Campus, Du Cane Road, London W12 0NN, UK.
[2]MRC Clinical Sciences Centre, Hammersmith Hospital Campus, Du Cane Road, London W12 0NN, UK.

In mammals, the onset of embryonic differentiation is accompanied by a dramatic series of cellular changes that completely transform the cells' transcriptional, epigenetic, metabolic and signalling landscape. Associated with these changes comes the potential for the emergence of abnormal or aberrant cells and how the embryo prevents these cells from contributing to further development, or the germline, remains largely unknown. Particularly problematic has been the study of those abnormal cells that carry defects that do not directly affect viability, but rather if they were to persist would be detrimental to the overall fitness of the organism. Over the last few years cell competition has emerged as a potential mechanism that can recognise and eliminate this type of cells. It is thought that cell competition acts by sensing relative fitness levels throughout a tissue, eliminating those cells deemed to be less fit than their neighbours. We have performed a screen for the pathways responsible for responding to these relative cellular fitness levels and identified the metabolic regulator mTOR. We find that in embryonic stem cells and mouse embryos mTOR signalling is a key read-out of cell-fitness levels during competition. We observe that mTOR pathway activity is decreased in less-fit cells specifically when they are confronted with their fitter counterparts, and that this loss of mTOR is sufficient to compromise cell viability. Furthermore, we show that this decrease in mTOR signalling is functionally important, as restoring mTOR activity in less-fit cells during competition, by shRNA knock-down of the mTOR inhibitor TSC2, is sufficient to rescue unfit cell elimination. Together these results suggest that during tissue growth, the signals that regulate fitness converge on a well-known metabolic determinant to enact winner and loser status, and that this process optimises the overall fitness of the developing tissue.

THIRD SESSION: APOPTOSIS, CELL COMPETITION AND CANCER. I

Regulation of apoptosis by IAPs and their antagonists

Hermann Steller
Rockefeller University. New York. USA.

The initiation of apoptosis in *Drosophila* requires the coordinated activity of Reaper, Hid and Grim (RHG) proteins. RHG proteins kill by inactivating Inhibitor of Apoptosis (IAP) proteins, the major known direct caspase inhibitors. RHG proteins are transcriptionally activated by many different pro-apoptotic signals, and this provides a mechanism by which different signaling pathways are integrated to initiate apoptosis. IAPs function as E3-ubiquitin ligase in the regulation of caspases and cell death in both *Drosophila* and mammals, and RHG proteins inactivate IAPs by stimulating their ubiquitination and degradation. Using mouse genetics, we showed that XIAP and one of its antagonists, ARTS, play critical physiological roles in the regulation of stem cell apoptosis, and that perturbation of this pathway has profound consequences for tumor development and wound healing. Furthermore, regulation of caspases by IAPs and their antagonists also controls the production of signals that apoptotic cells can release to profoundly affect their microenvironment. These include mitogenic signals that can help compensate for the loss of cells in response to tissue damage and injury ("compensatory proliferation") or, alternatively, pro-apoptotic signals that facilitate coordinated apoptosis ("apoptosis-induced apoptosis"). Collectively, this work revealed a conserved mechanism for negative regulation of caspases, established the physiological importance of natural IAP-antagonists for the initiation of apoptosis, and demonstrates the central role of the ubiquitin-proteasome pathway in this process.

ARTS protein regulates apoptosis and tumour suppression by causing degradation of XIAP and Bcl-2

Natalia Edison, Yael Curtz and Sarit Larisch
Biology Dept. University of Haifa.

ARTS (Sept4_i2) is a mitochondrial pro-apoptotic protein which promotes apoptosis by directly binding and degrading the two major proteins inhibiting apoptosis- X-linked Inhibitor of Apoptosis Protein (XIAP), and B-cell lymphoma 2 (Bcl-2). ARTS is localized to the outer mitochondrial membrane and initiates caspase activation upstream of mitochondrial outer membrane permeabilization (MOMP). ARTS antagonizes XIAP via a mechanism that is distinct from all other known IAP-antagonists. ARTS acts as a tumor suppressor protein in mice and humans. In particular, its expression is significantly reduced in leukemia, lymphoma and hepatocellular carcinoma (HCC) patients. Sept4/ARTS-Null mice show accelerated spontaneous tumor development, elevated XIAP

(but not cIAP1) levels, and increased numbers of stem cells that are resistant to cell death. Furthermore, the tumor and apoptosis phenotypes of *Sept4*/ARTS-deficient mice are suppressed by inactivation of XIAP, indicating that this protein is a major physiological target for the pro-apoptotic and tumor suppressor activity of ARTS. In addition, ARTS can act as a scaffold to bring XIAP into a complex with the anti-apoptotic Bcl-2 protein to stimulate degradation of Bcl-2 and promote mitochondrial apoptosis. Indeed, Mouse Embryonic Fibroblasts (MEFs) generated from *Sept4*/ARTS-deficient mice exhibit high levels of both Bcl-2 and XIAP, attesting to the role of ARTS as a novel Bcl-2 antagonist. Thus, ARTS functions as a dual antagonist of both XIAP and Bcl-2, two major negative regulators of apoptosis. As levels of both these anti-apoptotic proteins are high in many types of cancer, ARTS-based mimetics may be useful for cancer therapy.

Tumour development at the edge: p53 and cell competition

Ignacio Flores
Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC). Madrid. Spain.

Expansion of mutant cells during tumorigenesis leads to mosaic tissues in which clones of mutant cells are surrounded by non-mutant cells. One described example of this phenomenon is the presence of clones of keratinocytes harboring loss-of-function p53 mutations in skin areas exposed to sunlight. We have mimicked this process by grafting p53-null skin into the back of wild-type mice. Grafted animals develop $p53^{-/-}$ skin carcinomas with 100% penetrance. Importantly, every single tumor localizes at the border of $p53^{-/-}$ and wild-type regions and expands towards wild-type zones. Similarly, localized genetic deletion of p53 leads to increased tumor formation in areas composed of intermingled $p53^{-/-}$ and wild-type cells. Altogether, these results indicate that tumor susceptibility increases in transitional zones with differences in the levels of the tumor suppressor p53 and uncover a non cell-autonomous mechanism of tumor suppression.

FOURTH SESSION: APOPTOSIS, CELL COMPETITION AND CANCER. II

Signalling by apoptotic cells in development and cancer

Ainhoa Pérez-Garijo
Rockefeller University. New York. USA.

Apoptotic cells can produce signals to instruct cells in their local environment, including ones that stimulate proliferation. This apoptosis-induced proliferation has been shown to play important roles in tumor growth and relapse. We recently identified a novel mode of

communication by which apoptotic cells induce additional apoptosis in the same tissue. To investigate the role of apoptosis-induced apoptosis during development we studied the coordinated cell death that occurs in the adult wing of *Drosophila*. We observe a non-autonomous effect upon inhibition of apoptosis in the wing, suggesting there is a communication between dying cells to achieve synchronized cell death. Our results indicate there might be a wave of apoptosis traveling from the posterior compartment towards the anterior. Surprisingly, TNF and JNK pathways are not involved in this propagation of apoptosis, suggesting a different mechanism for apoptotic signaling in developmental and stress situations.

Role of apoptosis in cell competition and tumorigenesis

Ginés Morata
Centro de Biología Molecular Severo Ochoa. CSIC-UAM. Madrid. Spain.

Cell competition is an interactive process originally discovered in the imaginal discs of Drosophila. It is a mechanism that identifies and eliminates viable cells that are either not well adapted or have features that make them different from their neighbours. Part of this safeguard role is the removal of oncogenic cells that may appear during development. We are investigating this tumour-suppressing role of cell competition by studying in the wing disc of *Drosophila* the behaviour of groups of cells carrying oncogenic mutations like *scribble, disc large* or *rab5,* surrounded by non-tumour cells.

We find that that the general rule is that the oncogenic cells are eliminated by apoptosis mediated by the activity of the Jun N-Terminal Kinase (JNK) pathway. However, when the oncogenic cells form a group beyond certain size (around 500 cells) they successfully evade cell competition and develop an overgrowing tumour. Cell competition still functions in those tumours, but because it is a short-range mechanism, apoptosis is restricted to the border of the tumours and cells in the interior are protected. Under these conditions the permanent JNK activity generated by the cell competition at the borders stimulates further growth of the tumour due to proliferative signalling, involving the JAK/STAT and Wg/Dpp pathways, which emanate from the apoptotic cells. We are presently focused on the study of the mechanisms by which the JNK pathway induces over-proliferation. Our results indicate that cellular stresses administered to apoptosis-defective cells cause permanent JNK activity that in turn causes tumour overgrowths.

Simposio Internacional / *International Symposium*:

Competición celular, apoptosis y cáncer
Cell competition, apoptosis and cancer

Madrid, 25 y 26 de octubre de 2016 / *October 25-26, 2016*

ABSTRACTS

Cell competition in T lymphocyte development

Vera Martins
Gulbenkian Institute. Lisbon. Portugal.

Most T lymphocyte development occurs in the thymus from progenitors of bone marrow origin in a process characterized by high cellular turnover. We found that thymus turnover is regulated by cell competition. During T lymphocyte development, cell competition occurs at a specific stage of differentiation. At that stage, precursors differ slightly in their time of thymus residency. Specifically, 'young' hematopoietic precursors (that recently seeded the thymus) coexist with 'old' precursors (residing for longer in the thymus). These cells share the same surface markers that identifies them as sharing the same differentiation stage, but young precursors induce the clearance of the old, thereby promoting thymus turnover. This is a particularly dynamic process, as winner cells progressively differentiate into more mature stages. Impairment or disruption of cell competition is permissive to self-renewal and persistence of the old precursors. These maintain thymus function autonomously for some weeks with *de novo* production and export of T lymphocytes. However, prolongation of this property invariably leads to development of T cell acute lymphoblastic leukemia (T-ALL) with strong similarities to the human disease.

*Todos los derechos de propiedad intelectual son del autor. Queda prohibida la reproducción total o parcial de la obra sin autorización expresa del autor.
© FUNDACIÓN RAMÓN ARECES. Todos los derechos reservados.
**All intellectual property rights belong to the author. Total or partial reproduction of the work without express permission of the author is forbidden. © FUNDACIÓN RAMÓN ARECES. All rights reserved.*