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Meeting Report

Therapeutic targets in cancer cell metabolism and death

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Living standards have increased across the world. In particular, in strong economies, such as China, India and Brazil, a significant increase in life expectancy is likely to follow. The increase in the aged population is likely to result in an increased incidence of cancer. Although little in cancer treatment has changed in the last 20 years, significant progress has been made on comprehending the molecular mechanisms underlying the process of oncogenic transformation. We have learned that the cancer cell phenotype is achieved through the reactivation or alteration of existing cellular programmes used for normal cellular homeostasis. Such programmes coordinate essential processes, such as cell proliferation, migration, polarity, apoptosis and energy generation. Changes in energy metabolism between normal and cancer cells had been observed at the beginning of the twentieth century. It was noted that normal cells derive the majority of their energetic needs from ATP through oxidative phosphorylation in the mitochondria. In contrast, tumour cells exhibit the so-called Warburg effect, and they produce ATP even in the presence of oxygen through glycolysis, a catabolic pathway predominantly thought to be less efficient than oxidative phosphorylation. We now understand that one reason for this metabolic switch is that glycolysis provides the majority of the anabolic intermediates required to sustain high rates of cellular proliferation.

This conference focused on the recent developments in the understanding of the metabolic changes present in cancer cells and mechanisms leading to the resistance to programmed cell death in the process of oncogenic transformation. Such understanding is critical in the development of new therapeutic approaches for cancer. Researchers in this exciting field gathered for the meeting in Capri, Italy, where they discussed the most recent advances in cancer cell metabolism and death in an informal setting. The meeting started with a keynote lecture given by Craig Thompson (Philadelphia, PA, USA). Craig gave an exhaustive overview of what his and other laboratories have discovered in the last 10 years concerning the metabolic differences between normal and cancer cells. He introduced the concept of metabolic catastrophe as the main cause of oncogene-induced stress and apoptosis, suggesting that it derives from the uncoupled activation of the metabolic machinery without the adequate increase in a number of nutrient transporters. This concept was widely discussed throughout the meeting as the main source of oncogenic stress.

Oncogenic Alterations of Cellular Metabolism

Chi Van Dang (Baltimore, MD, USA) presented his latest work on the effect of c-Myc activation on cellular metabolism. His lab used a cellular model of B lymphoma, which involved P493 cells carrying a Tet-inducible Myc. In the P493 cells, the activation of Myc was sufficient to switch the metabolism towards aerobic glycolysis where most of the carbon derived from glucose results in lactate. Several years ago, his group found that lactic dehydrogenase (LDH) was a direct target of c-Myc. He recently confirmed this finding by performing Chip-Seq experiments and found that knocking down the expression of LDH-A strongly inhibited tumour formation. He also showed that FX11, a chemical inhibitor of LDH-A, induced cancer cell death, probably by forcing oxidative phosphorylation and subsequent reactive oxygen species (ROS) production.

As Craig highlighted in his introductory lecture, cancer cells cannot use glucose to provide their biosynthetic pathways with a source of reduced nitrogen. Cancer cells have solved this problem by increasing their glutamine uptake to feed the Krebs cycle. Again, c-Myc seems to be central in the coordination of glutamine metabolism. Chi Van Dang showed how c-Myc activation was able to induce mitochondrial biogenesis, which results in an increase in mitochondrial mass and glutamine metabolism. His lab recently found that one reason for this increase in glutamine metabolism is the upregulation of mitochondrial glutaminase (GLS), which

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is mediated by the c-Myc-dependent downregulation of miR23a/b that targets the 3′ UTR of the GLS messenger RNA. He described that under hypoxic conditions, cells increase glycolysis as expected, but they also seem to prefer glutamine and that the glutamate produced is preferentially used for glutathione production, leading to an increase in cellular reducing power. He also found that GLS inhibition conferred a greater sensitivity to apoptosis in cells carrying mutations in the isocitrate dehydrogenase *IDH1* gene. Craig extensively discussed the significant impact of mutations in IDH1 and 2 on cancer physiology. Mutations in IDH1 and 2 were initially discovered in glioblastoma and were later found to confer an altered catalytic activity to the encoded enzymes, leading to the conversion of isocitrate to the unique metabolite d2-hydroxyglutarate (d-2HG). Craig's lab analysed the d-2HG in the blood samples of leukaemia patients and found many of them to be positive for d-2HG. Interestingly, the patients carried mutations different than those found in glioblastoma. Still, the mutated enzyme had the same altered catalytic activity that produced d-2HG in glioblastoma. The possible analysis of cancer samples for their metabolite composition has tremendous prognostic potential and is becoming very attractive to biotech companies such as Metabolon, whose CEO, John Ryals (North Carolina, USA), was present at the workshop. He described the platform used by his company to perform metabolite screening, and he provided several practical examples of this analysis. Mutations in IDH1 seemed to result in a gain-of-function phenotype. Tak Mak's group (Toronto, Canada) decided, therefore, to explore this issue by using IDH1 mouse models. His lab produced both knockouts of IDH1 and knock-in mice carrying the mutation found in glioblastoma cells. Interestingly, the knockout mice were perfectly normal, whereas the knock-in animals died at the embryonic stage on day E10–11.

Although IDH1 and 2 mutations seem now to be linked to both glioblastomas and leukemias, Eyal Gottlieb (Glasgow, UK) pointed that there are only very few examples of metabolic genetic alterations that can be considered causative in cancer development. His presentation focused on the study of two cancer syndromes involving metabolic enzymes: hereditary leiomyomatosis and renal cell cancer (HLRCC) and hereditary paraganglioma and pheochromocytoma. These are caused by mutations in the genes encoding for fumatate hydratase (FH) and succinate dehydrogenase (SDH), respectively. Both FH and SDH enzymes work in the mitochondria and mediate the conversion of succinate into malate. It was shown that mutations in these two enzymes induce the accumulation of the intermediates succinate and fumarate that end-up inhibiting PHD activity and therefore stabilising Hif1. Eyal's group found that an analogue of α-ketoglutarate is able to restore low levels of Hif activity. This analogue seems to be effective in reducing kidney cyst size in a mouse model of HLRCC. Eyal also showed the results of a systems biology approach to identify pathways that could be synthetically lethal with FH mutations. Interestingly his group found that the haeme pathway might be one of those and that targeting the essential enzyme in haeme catabolism, haeme oxygenase 1 (Hmox1) could be a promising strategy to specifically kill cells with mutations in the *FH* gene.

Signalling Pathways and Metabolic Reprogramming

Cancers acquire alterations in several signal transduction pathways governing the way nutrients are used by the cell. The PI3K pathway is one of the most frequently mutated pathways in cancer. This pathway is known to give rise to tumours that have a significantly elevated glucose uptake and dependency. The PI3K pathway was, therefore, the subject of several talks. Almut Schulze (London, UK) discussed the Akt-dependent activation of genes involved in cholesterol and fatty acid biosynthesis. This activation is dependent on the SREBP transcription factor family. Activation of SREBP can be achieved by Akt through promoting SREBP translocation to the cell nucleus. She mentioned that this activation of SREBP downstream of Akt is mediated by the mammalian target of the rapamycin (mTOR) complex 1 (mTORC1) signalling pathway. Interestingly, she showed that blocking SREBP signalling downstream of Akt leads to the activation of the endoplasmic reticulum unfolded protein response (UPR^{ER}) and the concomitant activation of the PERK kinase and the transcription factor XBP.

Lewis Cantley (Boston, MA, USA) focused on a systems biology approach to elucidating the PI3K/Akt signal transduction pathway. He described how certain tumours in mice are addicted to the activation of the PI3k pathway for their survival. His group observed a strong regression in lung tumours driven by PI3K when treated with BEZ235, a synthetic PI3K inhibitor. He discussed how the PI3K pathway, one of the most frequently mutated pathways in cancer, gives rise to highly PET-positive tumours. This is due to the fact that PI3K/Akt has a dramatic effect on reprogramming metabolic networks for the enhancement of glycolysis through multiple targets, such as tuberin, FoxO and GSK3. He described how tumours reactivate PKM2, an embryonic form of pyruvate kinase, and downregulate PKM1, a more efficient form of this enzyme. This switch reduces the entry of pyruvate into the Krebs cycle and ensures a high cellular concentration of glycolytic intermediates heavily used by tumours as building blocks in the synthesis of nucleic acids and amino acids. His lab developed a method to compare the spectrum of metabolic intermediates in cells having only PKM1 or PKM2 or almost no PK activity. They observed that having no PK activity caused only a 40% decrease in the rate of glycolysis. This suggested the existence of an alternative method for producing pyruvate in the cells. He found that pyruvate can also be generated by the direct transfer of a phosphate group from phosphoenolpyruvate to the enzyme PGAM1.

The interconnections between signalling pathways and metabolism were also the subject of the talks by Eleanor Fish (Toronto, Canada) and Zachary Schafer (Indiana, USA). Eleanor Fish found that the chemokine CCL5 has a very complex effect on activated T cells. She described that CCL5 induces the activation of AMPK and GSK-3β, and she provided evidence that glycolysis was positively modulated on CCL5 engagement. Interestingly, 2-deoxy-glucose, a glycolysis inhibitor, also reduced CCL5-induced chemotaxis, suggesting a complex interconnection between metabolism and several aspects of cellular physiology. A similar degree of complexity was suggested by Zachary Schafer. He

found that the expression of ErbB2 in MCF10A rescued the cells from detachment-induced apoptosis (anoikis) because the expression of ErbB2 was able to restore ATP levels that were reduced by detachment. He also found that NADPH generated by the pentose phosphate pathway was essential for enabling ErbB2 to restore ATP levels and reduce cellular ROS, which increased on cell detachment.

Sensing Nutrients

Nutrients such as glucose and amino acids activate the mTOR pathway. This pathway controls processes, such as ribosome biogenesis and translational initiation, which are important for cell growth. The sterile-20-related kinase MAP4K3 has been recently proposed to function in a pathway that senses amino acid levels and activates the mTORC1. Richard Lamb (Alberta, Canada) described how phosphatase 2A subunit PR61ε negatively regulates the phosphorylation status and activity of MAP4K3 and, therefore, the output of the mTORC1 pathway. Richard also showed that in macrophages responding to Toll-like receptor (TLR) activation by LPS of the MEK–ERK pathway can also sense nutrients, particularly arginine. This effect seems to be mediated through the activation of another protein kinase, TPL-2. MAP4K3 has also been independently identified as an apoptosis inducer in an RNAi screen for modulators of DNA damage-induced apoptosis. L Miguel Martins (Leicester, UK) described how MAP4K3 modulates multiple signalling pathways to achieve the activation of BH3-only proteins and promote apoptosis in mammalian cells. He also reported that *Happyhour*, the *Drosophila* orthologue of *MAP4K3*, modulates apoptosis in flies by activating JNK signalling. A low cellular energy status inhibits mTORC1 by activating AMPK. Dario Alessi (Dundee, UK) described the mechanisms of AMPK activation by LKB1. His talk focused on the properties of the LKB1/STRAD/MO25 complex and discussed how the pseudo-kinase STRAD activates LKB1. Dario Alessi's talk introduced the interesting but nevertheless counterintuitive idea of how, in evolutionary terms, active kinases might have evolved from pseudo-kinases. One of the consequences of AMPK-dependent inactivation of mTORC1 is the activation of autophagy, a molecular recycling programme for impaired and defective cellular components. The importance of AMPK as a target for cancer treatment was also stressed by Stefano Indraccolo (Padova, Italy). His lab found that AMPK is activated in high glycolytic tumours treated with anti-VEGF antibodies and that the silencing of AMPK2 increased cell death in glucose-starved conditions. Eileen White (New Jersey, USA) described how the oncogene Ras upregulates autophagy and proposed that Ras-driven tumours need autophagy to support metabolism and survival. She suggested that this cellular process protects tumour cells by ensuring adequate mitochondria quality control. This process ensures adequate energy production in tumour cells through maintaining the efficiency of the Krebs cycle and the oxidative phosphorylation required for tumour growth. Xin Lu (Oxford, UK) focused on how tumours with mutations in the *RAS* oncogene utilise autophagy as a survival mechanism. Xin Lu's lab has characterised the ASPP family of proteins that interacts with p53. In her talk, she described how the ASPP family member ASPP2 is capable of potentiating Ras signalling. Interestingly, ASPP2 blocks autophagy in Ras-transformed cells. Xin Lu's talk provided more compelling evidence that autophagy is a cancer cell survival mechanism.

Sven Pettersson (Stockholm, Sweden) gave an interesting presentation that focused on the gut microbiota. He reminded the audience that each one of us carries approximately 1.5 kg of bacteria as part of our intestinal flora. He presented some interesting experimental data suggesting that manipulating the gut microbiota has significant effects on the cognition and behaviour of experimental rodents. The molecular basis for these effects seems to involve changes in cyclic AMP signalling triggered by the microbiota that in turn affect dopamine and serotonin pathways in the brain striatum. He also mentioned that the microbiota can signal to the host using the TLRs. He mentioned that this pathway of communication affects tumour formation in the Apc/Min mouse model of colon cancer.

ROS: The Nasty By-Products of Oxidative Phosphorylation

Activation of the PI3K/Akt pathway has the double effect of increasing glucose transporter membrane localisation and glucose utilisation, leading to the strong glucose dependency of cancer cells. However, even though glucose over-eating has a protective effect against apoptotic insults targeting mitochondria, it has the negative effect of increasing ROS production and DNA damage.

The importance of ROS production for normal and abnormal cell proliferation was analysed by several speakers. Navdeep Chandel (Chicago, IL, USA) discussed the need to uncouple the effect of mitochondrial ROS production from ATP generation to study the relative contribution of the two products of cellular respiration on cell physiology. He did this by altering the subcomponents of mitochondrial complex III by RNAi. Interestingly, he found that while knocking down the iron-sulphur 'Rieske' subunit impaired the production of both ROS and ATP, removing the QP-C subunit of complex III resulted in cells that still produced ROS but were impaired in ATP generation. Given that the knockdown of QP-C in primary fibroblasts prevented senescence although the fibroblasts lacking the Rieske subunit did undergo senescence, he concluded that low levels of ROS are needed to maintain the fitness of the cell. He also showed that ROS production was required for proper oxygen sensing during hypoxia; Hif stabilisation during hypoxia did not occur in cells that have lost the ability to produce mitochondrial-derived ROS following the knockdown of the Rieske subunit of complex III.

Mitochondrial oxidative phosphorylation has an important role in cancer physiology, as noted by Gyorgy Szabadkai (London, UK). He found that cisplatin (CDDP) treatment-induced metabolic reprogramming of the A549 lung cancer cell line that was associated with a decrease in glycolysis and an increase in oxidative phosphorylation. The excessive production of ROS associated with the increase in oxidative phosphorylation is probably one of the components of the cytotoxicity induced by CDDP. Therefore, he analysed the expression of several components of the mitochondrial respiratory chain and found that complex IV was upregulated.

Given that complex IV is encoded by mitochondrial DNA, he explored the mitochondrial DNA copy number and found it to be increased after CDDP treatment. Interestingly, in a CDDP-resistant variant of the A549 lung cancer cell line (CR), complex I was found to be mutated. Cells were deficient in oxidative phosphorylation if they did not compensate by inducing PGC1 expression and, therefore, increasing the mitochondrial copy number. Accordingly, he found that silencing PGC1β in CR cells increased their sensitivity to CDDP. Therefore, low levels of ROS have a positive effect on cellular proliferation and promote cancer transformation, but high levels can induce toxicity and have a tumour-suppressing effect. This concept was highlighted in some of the topics discussed by Tak Mak and Karen Vousden (Glasgow, UK). Tak Mak described how the protein DJ-1, also known as PARK7 because it is mutated in Parkinson's disease, is positively associated with protection against cancer. DJ-1 is known to protect neurons from oxidative stress-induced death. DJ-1 may have a similar action in cancer cells, and DJ-1 is activated by ROS in the absence of p53 and is also able to protect cancer cells against the effect of oxidative stress. Interestingly, Parkinson patients with mutations in DJ-1 have a 30% reduced chance of dying from cancer.

On the same theme, Karen Vousden suggested that the outcome of p53 induction following cellular stress can result either in cell survival or cell death depending on whether the p53 antioxidative action overcomes the pro-oxidative action. The choice between the pro- or anti-oxidative effects is dictated by the stress type, its intensity and context. In some cases, for instance, p53 can induce an adaptation to metabolic stress that induces genes coding for antioxidant proteins. One of the genes she discovered several years ago was *TIGAR*. *TIGAR* induction by p53 restrains glycolysis and promotes oxidative phosphorylation. By restraining glycolysis, glucose-6 phosphate is diverted towards the pentose phosphate shunt, increasing the production of cellular reducing power. TIGAR, therefore, protects cells from p53- and ROS-induced apoptosis. She found sequence similarity between TIGAR and the bisphosphatase domain of PFK2 (the FBPase-2 domain) and found that during hypoxia TIGAR translocates to the mitochondria where it binds to hexokinase 2. Interestingly, the loss of mitochondrial potential that occurs under hypoxic conditions is restored on TIGAR overexpression.

The talk given by Gerard Evan (Cambridge, UK) was centred on p53 as a stress sensor in cancer. Evan's laboratory created a knock-in gene replacement mouse model in which the endogenous p53 gene is substituted by one encoding p53ER. He has used this model to understand if p53 restoration in cancer would be an effective cancer cure. Among the plethora of stressors activating p53, oncogenic activation is well known. Oncogenic stress is supposed to activate p53 via ARF. However, oncogenes such as c-Myc are also induced during normal cellular proliferation without the activation of the ARF-p53 tumour-suppressing pathway; his group found that signal persistence is the factor that decides if c-Myc will activate the ARF-p53 pathway. Using a mouse model in which *KRAS* is driven by its own promoter, he found that p53 becomes activated only in high-grade regions, in only those regions where ARF is expressed. Therefore, he found no effect of p53 restoration on tumour regression in this mouse model. A few years ago, Gerard Evan created a mouse model to address the consequences of c-Myc inhibition *in vivo*. His group has shown that inducible inhibition of c-Myc in the entire mouse has the ability to induce cancer regression and has only mild and reversible side effects on proliferating tissues. The initial observation of tumour regression, however, showed that in $>50\%$ of the mice, tumour recurrence was induced once c-Myc inhibition ceased. He presented new data showing that episodic c-Myc inhibition is able to completely block recurrence.

Gerry Melino (Leicester, UK) discussed some preliminary results from his lab regarding the role of p63 and p73 in cancer and ageing. He reminded to the audience that the TAp73 knockout mice display a high incidence of tumours with a high rate of aneuploidy. At the molecular level, TAp73 physically binds kinetochore proteins such as Bub1, Bub3 and BubR1, de-regulating the phosphorylation of p55-cdc20 and therefore allowing premature entry into anaphase via APC/C. This mechanism seems to occur both in oocytes, leading to female infertility, and in cancer cells, leading to aneuploidy. Conversely, ΔNp73 knockout mice show a very reduced rate of spontaneous cancers. Gerry Melino described also results under way in a collaborative study with Tak Mak in Toronto in which they found that TAp73 knockout animals show an accelerated ageing phenotype with premature induction of cell senescence. Currently, the laboratory is evaluating both the possibility that senescence is related to changes in telomerase activity or in enhanced production of ROS. Gerry Melino reminded the audience that p53, another member of the same gene family, induces the expression of the mitochondrial phosphate-activated GLS type 2 (GLS2). These enzyme catalyses the hydrolysis of glutamine to glutamate and ammonia, increasing cellular glutamate steady state levels and decreased the levels of GSH, thus affecting intracellular ROS levels. This is a novel mechanism linking the p53 family to the regulation of metabolism.

Hypoxia and Metabolism

The availability of oxygen and nutrients is continuously provided to normal cells by the bloodstream. In the absence of neovascularisation, when a tumour mass starts to grow, its inner region immediately experiences a drastic reduction of both oxygen and nutrients. Adaptation to hypoxic cell growth implies a drastic change in cellular metabolism and, therefore, was the main topic of one of the workshop sessions. Lorenz Poellinger (Stockholm, Sweden) introduced the pleiotropic effect of hypoxia on many aspects of cellular and tissue physiology, including glucose metabolism, pH control, angiogenesis, matrix deposition and differentiation. He studied the effect of hypoxia on neuroblastoma and described how Hif1α is the principal mediator of an acute response to hypoxia, while Hif2α is associated with chronic hypoxia. His laboratory observed that neuroblastoma cells de-differentiated in hypoxic conditions to give rise to an immature neural crest-like phenotype. The process of de-differentiation seems to be both Hif- and Notch-dependent. The same integration of the Hif and Notch signalling pathways seems to be fundamental

for the hypoxia-associated EMT observed in different cancer cell lines. His laboratory found that once stabilised, Hif1α is able to interact with the Notch intra-cellular domain and enhance Notch function. Another observation made by his laboratory was that by analysing gene expression changes under hypoxic conditions in a number of different cancer cell lines, the most conserved gene expression signature was enriched not in metabolic genes, but rather in genes involved in transcriptional regulation. Among them, he focused on the jumonji protein JMJD1A and the histone methyltransferase G9a. The importance of G9a regulation by hypoxia in cancer might be underlined by the observation that G9a KO ES cells form smaller teratomas. Also, Peter Staller (Copenhagen, Denmark) presented his latest results on the role of the transcription factor FOXO3a during hypoxia in breast cancer and its importance in the adaptation to hypoxia. In contrast, Jacques Pouyssegur (Nice, France) focused on another aspect of hypoxia: metabolic acidosis. As a result of the altered metabolism of cells growing in the absence of oxygen, tumours experience a strong acidification of their micro-environment. Cancer cells, therefore, need to tightly control their intracellular pH to prevent intracellular acidification. He tested Ras-transformed fibroblasts carrying different silencing constructs for their ability to induce tumour formation in nude mice. The results showed that the $Na+/H+$ exchanger NHE1 was needed to induce tumour growth. Dependency on NHE1 is due to glycolysis, the main reason for acidification, because normal tumour growth is re-established by the concomitant silencing of the glycolytic enzyme phospho-glucose isomerase. Other proteins regulating intracellular pH are the carbonic anhydrases (CA) IX and XII. Interestingly, increased expression of CA IX induces cytosol alkalinisation, while silencing of both CA IX and XII strongly reduces tumour volume. Jacques Pouyssegur also showed that inhibition of the lactic acid transporter MCT1 was able to inhibit tumour growth. The same result was observed by silencing CD147, a chaperone that facilitates both MCT1 and MCT4 cell surface expression.

Cancer Prognosis and Therapy

Cancer progression is known to be a multistep process in which a series of genetic and epigenetic alterations accumulate to give rise to the final tumour. Ron DePinho (Boston, MA, USA) has proposed an episodic instability model to better describe the way a cell rapidly accumulates alterations during cancer genesis including prostate cancer. One of the problems in prostate cancer is that in many patients the disease never progresses to an aggressive stage. Yet many patients are unnecessarily subjected to aggressive treatment. He used a bioinformatic approach to address this issue in a mouse model with specific inactivation of PTEN in the prostate. This mouse develops prostatic intraepithelial neoplasia that never progress to an aggressive stage and is therefore a good model to find checkpoints crucial for cancer progression. His approach led to the identification of SMAD4, cyclin D1 and osteopontin as very good biomarkers to differentiate low- from high-risk prostate cancer patients. Another method to discriminate between different stages of malignancy was suggested by Laura Cerchia (Naples, Italy). She used Selex technology to find RNA aptamers that differentially bind to chemotherapy-resistant cancer cells with respect to their sensitive counterpart. Alternatively, Jan Paul Medema (Amsterdam, The Netherlands) addressed the cancer heterogeneity problem by examining cancer stem cells. The problem with cancer stem cells is that although they seem to be rare in the total cancer mass, they are resistant to the standard chemotherapeutic agents and are enriched after treatment. He found that different classes of HDAC inhibitors sensitise cancer stem cells to the toxic effect of oxaliplatin by driving cellular differentiation and potentially by upregulating Bmf and Bim.

Boris Zhivotovsky's (Stockholm, Sweden) talk focused on therapeutic approaches for neuroblastomas harbouring amplification of N-Myc. He described how α-tocopheryl succinate and other derivatives of vitamin E might act as promising candidates for the selective induction of apoptosis in neuroblastoma cells. This compounds triggered the accumulation of ROS in mitochondria, engaging the mitochondria-dependent apoptosis pathway.

Our Wish for Cancer Cells: (Apoptotic) Death

One of the main features of cancer cells is the acquired resistance against the engagement of cell death pathways such as apoptosis. Pascal Meier (London, UK) discussed the characterisation of mechanisms of apoptosis in the fruit fly *Drosophila melanogaster*, where caspase activation is under the strict control of the inhibitor of apoptosis proteins (IAPs). Degradation of IAPs in flies leads to spontaneous caspase activation and induction of classical apoptosis. In mammals, in which several IAP proteins are present, the issue is not so straightforward and the role of IAPs in blocking apoptosis is not so clear. Pascal's group has identified a novel cell death-inducing platform. This complex assembles on genotoxic stress and is formed independent of mitochondrial and death receptor pathways. Focused on the issue of apoptosis regulation by caspase-8, Doug Green (Memphis, TN, USA) reminded the audience that earlier studies suggested that caspase-8 not only promotes apoptosis but also protects cells from necrosis. Together, these results are somewhat confusing. Again, Doug mentioned that RIP1 and RIP3 are modulators of necroptosis inhibited by caspase-8. Although caspase-8 has a role in preventing apoptosis, the complex that is involved in the protection from necroptosis is a heterodimer of one molecule of caspase-8 and one molecule of FLIP. Doug suggested that the embryonic lethality of caspase-8, FADD or FLIP mice is suppressed by crossing any of these mutant mice with mice lacking RIP3. He showed that caspase-8/RIP3 double-mutant mice are viable but showed a progressive lympho-proliferative disease, which he suggested was due to a block in necroptosis. Many chemotherapeutic agents kill cells by blocking cell cycle progression through mitosis. Vishva Dixit (San Francisco, CA, USA) discussed cell death during prolonged mitotic arrest. He addressed whether the Bcl-2 family members may be involved in the cell death induced by mitotic arrest. He reported that the anti-apoptotic Bcl-2 family member Mcl-1 is rapidly degraded during mitotic arrest, sensitising cells to apoptosis. This sensitisation is mediated by proteasomal

degradation, and the E3 ligase responsible for the ubiquitination of Mcl-1 is the SCF family member Fbw7. This Fbw protein binds a phospho-degron in Mcl-1 that is produced soon after Cdk1 phophorylates Mcl-1, displacing PP2A and allowing the subsequent phosphorylation of the phospho-degron in Mcl1- by kinases belonging to the p38/JNK family.

Dead cells are normally removed through rapid engulfment (phagocytosis) by neighbouring cells. Michael Hengartner (Zurich, Switzerland) discussed the genetic determinants of cell engulfment in the nematode *Caenorhabditis elegans* (*C. elegans*). He described three signalling pathways that are essential for this process and their convergence on the GTPase Rac1. Michael Hengartner mentioned that the requirement of Rac1 for the process of engulfment is most likely due to the fact that this pathway promotes cytoskeletal rearrangements necessary for cell migration. The migration of a cell that is committed to engulfing a neighbouring cell corpse seems to be essential for efficient clearance of apoptotic cells. He explained how his group used engulfment-defective mutants to perform an RNAi screen for negative regulators of the engulfment process. Using this approach, srGAP, a GTPase-activating protein, was identified as a modulator of cell corpse persistence in *C. elegans*. His study underlined that the engulfment pathway is also used to eliminate damaged living cells. He speculated that this pathway could be an attractive opportunity to increase the efficiency of cancer treatments that act preferentially through damaging cancer cells.

Concluding Remarks

As cellular metabolism was one of the first differences found between normal and cancer cells, this workshop aimed to bring together scientists approaching tumourigenesis from different research fields that all support the belief that metabolic changes are a key aspect of cellular transformation. We believe that the workshop highly succeeded in its purpose. All the topics covered complemented each other to give a complete picture of the molecular basis of cellular transformation and tumourigenesis. Cancer metabolism became a key to the interpretation of oncogenic transformation that could help explain processes as different as cell death, proliferation and hypoxia adaptation. The workshop also extensively discussed the possibility to harness metabolic pathways to specifically dispose of cancer cells, and several promising targets were proposed.

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