Battling cancer on many fronts

Meeting on New Battlefields in Human Cancer—Attacking in Many Fronts

Fabian Zanella & Amancio Carnero⁺

Experimental Therapeutics Programme, Centro Nacional de Investigaciones Oncológicas (CNIO), Madrid, Spain

The Spanish National Cancer Centre (CNIO)—Oncotrain Meeting on New Battlefields in Human Cancer—Attacking in Many Fronts took place between 10 and 11 March 2008, at the CNIO in Madrid, Spain, and was organized by eight graduate students and financed by Marie Curie Initiatives at the CNIO.

Keywords: cancer; cell cycle; drug development; cancer stem cells

Cycling to cancer: cell cycling and cell division

Cancer is ultimately a cell-cycle disease as most tumours occur owing to one or more errors that derail the cell-cycle machinery. Such defects can affect either components of the cell cycle itself—including checkpoint mechanisms—or elements of upstream signalling cascades, which should ultimately converge to trigger cell-cycle events (Malumbres & Carnero, 2003). These cell-cycle aberrations not only damage the regulation of the G1-to-S phase, but also alter the mitotic checkpoint, which gives rise to the chromosomal alterations observed in most human tumours.

E. Nigg (Martinsried, Germany) reviewed the mitotic checkpoint and then focused our attention on his recent work with PLK1 and PLK1-interacting checkpoint helicase (PICH). PLK1 is a crucial regulator of mitotic progression and cell division in eukaryotes. It is highly expressed in tumour cells and is considered to be a potential target for cancer therapy. By using ZK-thiazolidinone (TAL), which is a small-molecule inhibitor of mammalian PLK1, Nigg has studied the role of PLK1 in sister-chromatid separation, centrosome maturation and spindle assembly, and has shown that PLK1 activity is essential for cleavage-furrow formation and regression, leading to successful cytokinesis (Santamaria *et al*, 2007). PICH is a member of the SW1/SNF2 family of DNA-dependent ATPases and is an essential component of the spindle-assembly checkpoint. It has been shown to localize to kinetochores, inner centromeres and the thin threads that connect separating chromosomes during anaphase (Baumann *et al*, 2007). PICH associates with centromeric chromatin during anaphase, and the analysis of PICH-positive anaphase threads has shown that they comprise mainly alphoid centromere DNA. Most PICH-positive threads evolve from inner centromeres, as these stretch in response to tension, suggesting that PICH might be a sensor of tension during chromosome separation (Baumann *et al*, 2007).

M. Malumbres (Madrid, Spain) discussed the work of his group with the APC/C, which is a ubiquitin ligase that targets mitotic substrates for degradation in complex with either CDC20 or CDH1 co-factors. The specificity of the complex for certain substrates is determined by the cofactor involved, and the Malumbres group has studied this specificity by generating conditional knockout (KO) models of both proteins in mice. Their work has shown that CDC20 is essential for embryo development, and that its absence arrests development at the two-cell embryo stage at the point of mitosis, perhaps owing to a large accumulation of cyclin B (Fig 1).

EMBO reports (2008) 9, 853–858. doi:10.1038/embor.2008.140

See Glossary for abbreviations used in this article.

Introduction

In March 2008, around 100 young scientists met at the Spanish National Cancer Centre (CNIO) in Madrid, Spain, to share their results with one another and a few invited senior scientists from their fields. The workshop was organized by eight foreign students on the Oncotrain programme—supported by the VI European Framework—who are undertaking their PhDs at the CNIO in the fields of cancer research. As such, the meeting reflected the areas in which the organizers are working, namely cell cycle, cancer genetics, drug discovery and cancer stem cells (CSCs). Posters and oral presentations were grouped around these areas, with two key lectures and a few selected oral presentations per session.

Experimental Therapeutics Programme, Centro Nacional de Investigaciones Oncológicas (CNIO), Melchor Fernández Almagro, 3, 28029 Madrid, Spain

⁺Corresponding author. Tel: +34 917328000; Fax: +34 917328051;

E-mail: acarnero@cnio.es

Submitted 10 April 2008; accepted 23 June 2008; published online 8 August 2008

Glossary

ABL1	v-abl Abelson murine leukaemia viral oncogene homologue 1
ALK	anaplastic lymphoma kinase
APC/C	anaphase-promoting complex/cyclosome
ATIC	5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase
BRCA	breast cancer susceptibility gene
CDC20	cell-division cycle 20
CDH1	CDC20 homologue 1
CHK1	checkpoint kinase 1
FOXO3a	forkhead box O3a
HIV	human immunodeficiency virus
HPV16	human papillomavirus 16
HSP90	Heat-shock protein 90
IL-3	interleukin-3
LYPLA2	lysophospholipase II
MAPK	mitogen-activated protein kinase
miRNAs	microRNAs
MMP	matrix metalloproteinase
MT1	membrane type 1
OCT4	octamer-binding transcription factor 4
PEDF	pigment epithelium-derived factor
PI(3)K	phosphatidylinositol-3-kinase
PLK1	Polo-like kinase 1
RAI	retinoic acid-inducible
REV	regulator of virion expression
RPL5	ribosomal protein L5
STAT5	signal transducer and activator of transcription 5
SNF2	sucrose nonfermentable 2
SW1	switch 1
WNT3	wingless-type MMTV integration site family member 3

Similarly, *CDH1-KO* embryos die after progression to embryonic day 10.5. The *CDH1-KO* embryos showed decreased haematopoiesis and important defects in maternal–embryo blood exchange in the placenta. CDH1 is important for the endoreduplication of cells. The absence of CDH1 in the placenta and embryo reduced the size of the nucleus of placenta trophoblast giant cells; by contrast, the specific ablation of CDH1 in the embryo rescued the phenotype and the embryos survived, although the mice died shortly after birth. In CDH1-null cells, most APC/CDH1 targets are upregulated, which results in inefficient DNA replication and genomic instability. It is possible that these defects in DNA replication and genomic instability explain the reduced tumour formation after carcinogenic treatment in mice with only one allele of *Cdh1*.

How could genetics explain cancer?

Cancer is a genetic disease in which the evolution from normal to cancerous tissue is driven by genetic—including cytogenetic—or epigenetic changes. Nowadays, new genetic and cytogenetic tools allow mass analysis and identification of candidate regions and genes that are involved in disease. However, these alterations do not affect all individuals equally and genetic background is important. These tools also allow for the identification of single-nucleotide polymorphisms (SNPs) that confer a susceptibility to cancer. The identification of these SNPs and the way in which they interact is essential to understanding cancer risks in some individuals. J. Benitez (Madrid, Spain) presented data to show how his group distinguishes between low-penetrance and moderate-penetrance

genes, which confer susceptibility to sporadic cancer, and high-penetrance genes, which are responsible for hereditary cancer. His group has identified a 1-Mb region of chromosome 21q that probably contains a high-penetrance gene member of one of the non-BRCA1/BRCA2 families. The Benitez group also found two SNPs of the vitamin D receptor that is associated with differentiation grade and tumour aggressiveness in non-related sporadic breast cancer.

Comparative genomic hybridization (CGH) was developed to detect chromosomal copy-number changes on a genome-wide scale. Using arrays to perform CGH (CGHa) allows the detection of chromosome copy numbers at a high-resolution scale (Ijssel & Ylstra, 2007; Ylstra *et al*, 2006). By using CGHa, B. Ylstra (Amsterdam, The Netherlands) and collaborators found small chromosomal regions responsible for many tumour phenotypes (Jong *et al*, 2007; Buffart *et al*, 2007; Paterson *et al*, 2007). In an attempt to identify novel non-small-cell lung cancer (NSCLC)-related genes, they performed genome-wide screening of chromosome copy number in stage I and II tumours. They identified a deletion on 14q32-33 that correlated with increased survival. HSP90 resides in that region, and low levels of HSP90 expression were correlated with increased survival (Gallegos Ruiz *et al*, 2008). Ylstra also presented chromosomal alterations that allow differentiation between HPV16-induced and environmental carcinogenesis-induced head and neck squamous-cell carcinoma (HNSCC). Four regions showed differences between HPV16-positive and HPV16-negative tumours, suggesting that these regions were involved in the initial stages of tumorigenesis (Smeets *et al*, 2006). Seven other chromosomal regions have common alterations in both types of tumour, suggesting involvement in later stages of HNSCC development.

A collaboration between P. Asero (Catania, Italy) and T. Visakorpi (Tampere, Finland) has resulted in the identification of target genes for the common 1p amplification found in bladder cancer. Using CGH, they have narrowed down the location of the amplification to a 1-Mb region between 1p21-22 and 1p35-36. Asero and Visakorpi suggested that the increased levels of expression of RPL5, acid82K, FLJ13150, CGI100, GALE and LYPLA2 associated with the cancer are due to their roles as putative oncogenes. A.E. Rodriguez (Salamanca, Spain) identified genomic abnormalities in B-cell chronic leukaemia (CCL) by CGH (gains of 13q, 17p, 11q and 12q). With respect to the 12q gain, his group initially identified it as a minimal 12q12-q24 gain, determined the genes comprised within this region and evaluated their expression in the transcriptome of samples from CCL patients. They found increased expression of 144 genes associated with CCL, of which 92 were located in the 12q12-q24 region, confirming that chromosomal gains or losses might reflect the imbalance of transcription found in tumour cells, as the chromosomal changes paralleled the expression changes of genes within the region (Rodriguez *et al*, 2007). G. Gundrem (Barcelona, Spain) presented an improved and extendable database that integrates microarray-expression data and CGH arrays in specific tumour types to allow transcriptomic, large-scale genomic data and somatic mutation data analyses (www.intogen.org).

M. Scatolini (Valenta, Italy) presented the results of a transcriptomic analysis of the various stages of melanoma progression. Her comparison of the signatures of the intermediate steps of the process showed that Notch1 pathway deregulation seems to be essential in the transition between the common nevi and primary radial growth-phase melanomas. The transition between these lateral and vertical growth-phase melanomas is characterized by the alteration of WNT3, MAPK and AKT pathways. Interestingly, dysplastic nevi do not seem to be an intermediate step in the nevo–melanoma transition.

Fig 1 | Embryonic lethality caused by the genetic ablation of the APC/C cofactors, CDC20 mutant or CDH1 in the mouse. Picture kindly provided by M. Malumbres (Spanish National Cancer Centre, Spain). APC/C, anaphase-promoting complex/cyclosome; CDC20, cell-division cycle 20; CDH1, CDC20 homologue 1; E, embryonic day; P, postnatal stage.

Surprisingly, only a few presentations discussed miRNAs. The studies presented by M.J. Bueno (Madrid, Spain) and A.B. Martínez-Cruz (Madrid, Spain) were of note. Bueno used a genetic analysis of mouse tumours to identify miRNAs involved in tumorigenesis, and showed that the genetic and epigenetic silencing of miR-203 might enhance ABL1 and BCR-ABL1 oncogene expression in haematological malignancies (Bueno *et al*, 2008). In fact, miR-203 functions as a tumour-suppressor gene, and its silencing by promoter hypermethylation is involved in lymphoid and myeloid malignancies. Martínez-Cruz compared the deregulation of miRNAs in various mouse models displaying distinct susceptibility to spontaneous epithelial tumours. The differential pattern of miRNA expression allowed the identification of miRNAs that were possibly responsible for such susceptibility and could be used to predict various tumour characteristics in the future. It seems likely that the identification of miRNAs that are associated with each genetic or epigenetic alteration, the analysis of the behaviour of different miRNAs in tumours, and the role of each miRNA in downregulating specific targets will constitute important research topics in cancer genetics.

Structures and drug development

P. Workman (London, UK) comprehensively introduced the process of drug discovery. To illustrate each point, Workman explored in detail the process that led to the discovery and development of HSP90 inhibitors (for a review, see Workman, 2003, 2004; McDonald *et al*, 2006). Linking drug discovery with the mitotic checkpoint, M. Zajac (Madrid, Spain) and B. Martínez-Delgado (Madrid, Spain) showed that BRCA1 mediates the G2/M transition mainly through CHK1

when treated with the HSP90 inhibitor 17AAG. On treatment with the drug, the cells arrested at the G2/M phase and entered aberrant mitosis in a BRCA1-dependent manner. A failure to arrest the cells at or before mitosis resulted in the formation of micronucleated cells, the aberrant segregation of chromosomes, microtubule misalignment and multicentrosomes, eventually leading to mitotic catastrophe and cell death.

J. Bravo (Madrid, Spain) reviewed the process of drug discovery from a structural point of view and explained how developing the crystal structure of any given target could help in the drug-discovery process. G. Bardenes (Valencia, Spain) presented an approach to drug discovery using protein–protein interaction inhibitors through structure-based drug design. In addition, Bardenes reported on some work that aimed to identify inhibitors of heparanase and MT1-MMP—enzymes that degrade extracellular matrix components, which are increased in some tumours—by using nuclear magnetic resonance (NMR). F.E. Boccalatte (Torino, Italy) presented the results from a functional phosphoproteomic analysis of anaplastic large-cell lymphoma (ALCL), and showed that ALK-mediated ATIC phosphorylation enhances ATIC enzymatic activity, thereby hampering methotrexate-mediated transformilase-activity inhibition, and suggesting ALK as a new therapeutic target for ALCL, in combination with methotrexate. S. Numanoglu (Izmir, Turkey) explored the effect of hyperthermia in doxorubicin-treated human breast carcinoma cells. Hyperthermia causes free radicals that induce DNA breaks, mutations and apoptosis, and, its combination with a topoisomerase I inhibitor as doxorubicin, increases its cytotoxic activity.

Fig 2 | Schematic overview of the BaFiso assay system. (A) Paired isogenic cell lines engineered to acquire interleukin 3 (IL-3)-autonomous growth through constitutive activation of AKT or STAT5 signalling. The two cell lines are individually tagged with either yellow or cyan fluorescent proteins. The deep-red fluorescing agent DRAQ5 was used to perform automated segmentation of cell nuclei (red cells). (B) Isogenic cells were co-cultured and treated with compounds, and the change in the relative cell number was calculated by distinct fluorescence. (C) Image representative of the co-culture of isogenic BAF3 lines expressing distinct fluorescent markers. Image kindly supplied by W. Link (CNIO, Spain). RTK, receptor tyrosine kinase; STAT5, signal transducer and activator of transcription 5.

F. Zanella (Madrid, Spain) presented interesting cell-based assays that were developed for the identification of new anti-tumoural compounds. To identify small-molecule inhibitors of the PI(3)K pathway, a high-throughput cell-imaging assay was established to monitor the nucleo-cytoplasmic translocation of a FOXO3a-GFP (green fluorescent protein) fusion protein in tumour cells (Zanella *et al*, 2008). Another cell-based screening platform was developed to identify nuclear-export inhibitors and specific silencers of the nuclear-export machinery using a GFP-labelled REV protein from HIV. This platform was also shown to be useful as a counter screen for pathway deconvolution (Zanella *et al*, 2007). Finally, another system based on the genetically engineered IL-3-dependent Ba/F3 cells was shown. In these cells, deprivation of IL-3 induces death, which can be overridden by the activation of the PI(3)K, RAS or STAT5 pathways. On the basis of this information, a dual fluorescence-based platform was generated to explore differential cell survival using multiparametric cell imaging (Rosado *et al*, 2008), which allows the identification of selective inhibitors specific for PI3K (Fig 2).

As targeting CSCs apparently holds some hope for improving anticancer therapies, R. Fernandez-Alonso (Leon, Spain) reported a cellular system to identify compounds with the ability to target CSCs. Using GFP under the control of the OCT4 promoter, which was ectopically expressed into embryoid bodies, the investigators were able to regulate the levels of expression of GFP and to monitor the behaviour of the stem cells of the embryoid body. Fernandez-Alonso screened a chemical library of marine compounds and identified regulators of the expression of OCT4. As OCT4 is one of the crucial regulators of stem-cell self-renewal, therapies that regulate this protein are expected to have a direct effect on the response of the tumours.

The stem of cancer

The last session of the workshop was dedicated to CSCs. The current model of carcinogenesis describes the formation of a tumour by the sequential accumulation of mutations in oncogenes and tumour-suppressor genes. Tumours are therefore formed from a heterogeneous population of cells, the members of which continue to acquire new mutations with the clones selectively amplified by pure Darwinian selection. The current hypothesis of CSCs states that only a small and distinctive proportion of the cancer cells can propagate the tumour. The CSCs constitute this small subpopulation of cancer cells, the members of which have characteristics that are normally associated with stem cells. The definition of a CSC does not necessarily imply its origin from a stem or progenitor cell,

although current trends to identify them are based on the hypothesis that CSCs express surface markers that are associated with immature cell types. The identification of correct CSC markers has become a central, almost circular, task: it is necessary to identify reliable markers of CSCs to identify the subpopulation of CSCs.

M.A. Blasco (Madrid, Spain) explored the role of telomeres and telomerase in stem-cell biology. Using confocal telomere quantitative-fluorescence *in situ* hybridization (telomapping), the Blasco laboratory has found gradients of telomere length within tissues, with the longest telomeres mapping to the known stem-cell compartments. The cells with the longest telomeres behave as stem cells on treatment with mitogenic stimuli (Flores *et al*, 2008). Therefore, relative telomere length can be used as a stem-cell marker. Telomeres shorten with age in various mouse stem-cell compartments, which parallels a decline in stem-cell functionality, indicating that telomere loss might contribute to stem-cell dysfunction with age (Flores *et al*, 2008). The next step is the use of the telomapping technique to explore the presence and behaviour of CSCs. In mice without telomerase activity (*Terc*^{-/-}), p53 is increased, and the combined absence of *Terc* and p53 restores the mobilization of stem cells. Blasco proposed a general model in which p53 controls the checkpoint preventing stem cells with crucially short telomeres to regenerate aged tissue; however, the absence of p53 allows stem cells to form tumours (Finkel *et al*, 2007; Matheu *et al*, 2007). Therefore, telomere length is rate limiting for mouse aging, owing to an inability to restore aged/damaged tissue. It seems possible that the increase in telomerase activity, which prevents the appearance of tumours, could increase the lifespan of mice. To address this issue, in collaboration with M. Serrano (Madrid, Spain), Blasco crossed mice with increased telomerase activity in the skin (*K5-Tert*) with mice carrying an extra copy of the p53, p16INK4a and p19ARF loci (*super-p53/INK4*), which are known to be more resistant to cancer (Garcia-Cao *et al*, 2002, 2006; Matheu *et al*, 2007). These mice showed decreased symptoms of ageing and increased cancer survival.

Neurogenesis is known to occur in the specific niches of the adult mammalian brain; however, whether germinal centres exist in the neural crest-derived peripheral nervous system is unknown. R. Pardal (Sevilla, Spain) and co-workers have discovered stem cells in the adult carotid body, which is an oxygen-sensing organ of the sympathoadrenal lineage that grows in chronic hypoxaemia. Pardal showed that carotid body growth during hypoxia, and the production of new neuron-like glomus cells, results from the activation of a resident population of neural crest-derived progenitors. On returning to normoxia, the carotid body size is restored and approximately 50% of the carotid body glomus cell mass is replaced by the newly formed cells, indicating a notable regenerative power that is unusual in an adult neural tissue. *In vivo* and *in vitro* analyses indicate that carotid body stem cells self-renew and are multipotent. Pardal also provided compelling evidence that glia-like type II cells are the *in vivo* precursors of new glomus cells, and demonstrated that the newly formed glomus cells have the same complex neurochemical and electrophysiological properties as the *in situ* carotid body (Pardal *et al*, 2007). Several other groups focused on the central nervous system to identify CSCs and markers regulating their properties. Using *p73-KO* mice, L. Gonzalez-Cano (Tenerife, Spain) showed that p73, which is a member of the p53 family, is a positive regulator of embryonic stem-cell renewal. B. Ortensi (Milan, Italy) studied the role of the *Rai* gene in CSCs

isolated from glioblastoma multiforme tumours. *RAI* is a neuronal-specific member of the family of the Shc-like adaptor proteins that functions as a neuroprotective factor in mature neurons by activating the PI(3)K pathway (Villanacci *et al*, 2008). *RAI* levels are increased in progenitor cells but decreased in differentiating cells. *RAI* promotes neurogenesis through the β -catenin pathway and is expressed specifically in glioblastoma multiforme tumours. It will be interesting to test the effect of silencing *RAI* in tumours, as *Rai*^{-/-} cells have impaired differentiation and no migration ability.

At the molecular level, the alteration of stem-cell renewal pathways and/or the inhibition of differentiation processes have been recognized as essential steps for the transformation of CSCs. However, based on oncogene-induced plasticity, it has also been proposed that CSCs could be the result of a de-differentiation process induced by the expression/inhibition of a specific combination of genes (Rapp *et al*, 2008). Given the large heterogeneity of tumours, it is possible for both hypotheses to be correct.

Finally, there is a theory that such cells persist in tumours as a distinct population, and cause relapse and metastasis by giving rise to new tumours. The development of specific therapies targeted at CSCs holds hope for improving the survival and quality of life of cancer patients, especially sufferers of metastatic disease. C. Gil (Albacete, Spain) and C. Ramirez-Castillejo (Albacete, Spain) identified PEDF, which is a secreted factor that promotes the self-renewal of neural stem cells *in vitro* (Ramirez-Castillejo *et al*, 2006). Treatment of different cellular neuronal subpopulations reveals various susceptibilities to chemotherapy. Combining standard chemotherapy with the carboxy-terminal fragment of PEDF alters the response to treatments and could lead to new strategies to abolish CSC-derived resistance.

Concluding remarks

The Oncotrain meeting could be seen as representing a small survey of the 'temperature' of various areas of cancer research. There was an emphasis on certain subjects—the regulation of the mitotic checkpoint and the relevance of various genes to cancer, for example—and it was clear that there is still much to be discovered. In addition, although the analysis of genetic and cytogenetic alterations on a massive scale is being applied to large numbers of samples in order to identify prognostic markers, more functional studies are necessary. Finally, there is no doubt that a better understanding of CSCs will lead to a new era of both basic and clinical cancer research, the reclassification of human tumours and the development of novel therapeutic strategies specifically targeting CSC.

All in all, this was an exciting and successful meeting, which was wonderfully organized by the Oncotrain students with the help of the Scientific Events Office of the CNIO. This format should be repeated, because it brings together established and fresh scientific blood in a friendly, scientific environment that allows the students to discuss their work and exchange ideas.

ACKNOWLEDGEMENTS

Work in the laboratory of A.C. is funded by the Fundacion Mutua Madrileña, the Spanish Ministry of Education and Science (SAF2005-00944) and the VI Framework of the European Commission (Project Netsensor).

REFERENCES

- Baumann C, Korner R, Hofmann K, Nigg EA (2007) PICH, a centromere-associated SNF2 family ATPase, is regulated by Plk1 and required for the spindle checkpoint. *Cell* **128**: 101–114
- Bueno MJ, Pérez de Castro I, Gómez de Cedrón M, Santos J, Calin GA, Cigudosa JC, Croce CM, Fernández-Piqueras J, Malumbres M (2008)

- Genetic and epigenetic silencing of microRNA-203 enhances ABL1 and BCR-ABL1 oncogene expression. *Cancer Cell* **13**: 496–506
- Buffart TE *et al* (2007) Gastric cancers in young and elderly patients show different genomic profiles. *J Pathol* **211**: 45–51
- Finkel T, Serrano M, Blasco MA (2007) The common biology of cancer and ageing. *Nature* **448**: 767–774
- Flores I, Canela A, Vera E, Tejera A, Cotsarelis G, Blasco MA (2008) The longest telomeres: a general signature of adult stem cell compartments. *Genes Dev* **22**: 654–667
- Gallegos Ruiz MI *et al* (2008) Integration of gene dosage and gene expression in non-small cell lung cancer, identification of HSP90 as potential target. *PLoS ONE* **3**: e0001722
- Garcia-Cao I, Garcia-Cao M, Martin-Caballero J, Criado LM, Klatt P, Flores JM, Weill JC, Blasco MA, Serrano M (2002) “Super p53” mice exhibit enhanced DNA damage response, are tumor resistant and age normally. *EMBO J* **21**: 6225–6235
- Garcia-Cao I, Garcia-Cao M, Tomas-Loba A, Martin-Caballero J, Flores JM, Klatt P, Blasco MA, Serrano M (2006) Increased p53 activity does not accelerate telomere-driven ageing. *EMBO Rep* **7**: 546–552
- Ijssel P, Ylstra B (2007) Oligonucleotide array comparative genomic hybridization. *Methods Mol Biol* **396**: 207–221
- Jong K *et al* (2007) Cross-platform array comparative genomic hybridization meta-analysis separates hematopoietic and mesenchymal from epithelial tumors. *Oncogene* **26**: 1499–1506
- Malumbres M, Carnero A (2003) Cell cycle deregulation: a common motif in cancer. *Prog Cell Cycle Res* **5**: 5–18
- Matheu A *et al* (2007) Delayed ageing through damage protection by the Arf/p53 pathway. *Nature* **448**: 375–379
- McDonald E, Workman P, Jones K (2006) Inhibitors of the HSP90 molecular chaperone: attacking the master regulator in cancer. *Curr Top Med Chem* **6**: 1091–1107
- Pardal R, Ortega-Saenz P, Duran R, Lopez-Barneo J (2007) Glia-like stem cells sustain physiologic neurogenesis in the adult mammalian carotid body. *Cell* **131**: 364–377
- Paterson AL *et al* (2007) Co-amplification of 8p12 and 11q13 in breast cancers is not the result of a single genomic event. *Genes Chromosomes Cancer* **46**: 427–439
- Ramirez-Castillejo C, Sanchez-Sanchez F, Andreu-Agullo C, Ferron SR, Aroca-Aguilar JD, Sanchez P, Mira H, Escribano J, Farinas I (2006) Pigment epithelium-derived factor is a niche signal for neural stem cell renewal. *Nat Neurosci* **9**: 331–339
- Rapp UR, Ceteci F, Schreck R (2008) Oncogene-induced plasticity and cancer stem cells. *Cell Cycle* **7**: 45–51
- Rodriguez A *et al* (2007) Molecular heterogeneity in chronic lymphocytic leukemia is dependent on BCR signaling: clinical correlation. *Leukemia* **21**: 1984–1991
- Rosado A, Zanella F, Garcia B, Carnero A, Link W (2008) A dual-color fluorescence-based platform to identify selective inhibitors of Akt signaling. *PLoS ONE* **3**: e1823
- Santamaria A *et al* (2007) Use of the novel Plk1 inhibitor ZK-thiazolidinone to elucidate functions of Plk1 in early and late stages of mitosis. *Mol Biol Cell* **18**: 4024–4036
- Smeets SJ, Braakhuis BJ, Abbas S, Snijders PJ, Ylstra B, van de Wiel MA, Meijer GA, Leemans CR, Brakenhoff RH (2006) Genome-wide DNA copy number alterations in head and neck squamous cell carcinomas with or without oncogene-expressing human papillomavirus. *Oncogene* **25**: 2558–2564
- Villanacci V, Bassotti G, Ortensi B, Fisogni S, Cathomas G, Maurer CA, Galletti A, Salerni B, Pelicci G (2008) Expression of the Rai (Shc C) adaptor protein in the human enteric nervous system. *Neurogastroenterol Motil* **20**: 206–212
- Workman P (2003) Overview: translating Hsp90 biology into Hsp90 drugs. *Curr Cancer Drug Targets* **3**: 297–300
- Workman P (2004) Combinatorial attack on multistep oncogenesis by inhibiting the Hsp90 molecular chaperone. *Cancer Lett* **206**: 149–157
- Ylstra B, van den Ijssel P, Carvalho B, Brakenhoff RH, Meijer GA (2006) BAC to the future! or oligonucleotides: a perspective for micro array comparative genomic hybridization (array CGH). *Nucleic Acids Res* **34**: 445–450
- Zanella F, Rosado A, Blanco F, Henderson BR, Carnero A, Link W (2007) An HTS approach to screen for antagonists of the nuclear export machinery using high content cell-based assays. *Assay Drug Dev Technol* **5**: 333–341
- Zanella F, Rosado A, Garefa B, Carnero A, Link W (2008) Chemical genetic analysis of nuclear-cytoplasmic shuttling of FOXO using image-based cell screening. *Chem Biol* **8**: (in press)

Fabian Zanella

Amancio Carnero