tumourigenesis is evident in the observed resistance of cyclin D1 knockout mice to the development of breast cancer.

The cyclin D1/CDK complexes also act as a point of integration between growth factor signalling pathways and the cell cycle. This is exemplified by the fact that expression of cyclin D1 is under both transcriptional and post-translational control in response to cellular signals. In particular, turnover of cyclin D1 protein is a highly regulated process that can be activated through phosphorylation at Threonine 286 (T286), which triggers nuclear export and ubiquitin-dependent degradation of cyclin D1 via the 26S proteosome.

We have become particularly interested in the control of cyclin D1 turnover, which is likely to present novel anti-cancer therapeutic targets in malignancies over-expressing this protein. With this in mind, we have been investigating the regulation of T286 phosphorylation on cyclin D1. From these studies we have identified a novel pathway through which this event is modulated, leading to 26S proteasome-dependent degradation of cyclin D1. This pathway will be described, along with potential therapeutic consequences for cancer treatment.

385

Cancer therapy based on p53 and Rb

M. Korn, *UCSF Comprehensive Cancer Center & Cancer Research Institute, San Fransisco, USA*

The majority of malignancies harbor molecular alterations disabling the tumor-suppressor function of the p53 and Rb pathways. Inactivation of these pathways is also essential for efficient replication of human adenovirus. Exploiting this analogy, adenovirus mutants have been created that replicate selectively in cancer cells with alterations of these pathways. For example, ONYX-015 is an adenovirus mutant selectively replicating in tumor cells with inactivated p53 pathway. This virus lacks the E1B55k gene, which is necessary for inactivation of p53, thus restricting virus replication to cells that lost p53-function. Similarly, ONYX-411 harbors, among other features, a deletion within the E1A gene and targets cells with deregulated E2F resulting from mutations affecting the Rb pathway. Clinical studies demonstrated that intratumoral, intraarterial, and intravenous injection of ONYX-015 is safe. In particular in combination with standard chemotherapeutic agents, evidence of anti-tumor activity of this virus was found in clinical trials in patients with cancer of the head- and neck and gastrointestinal cancer metastatic to the liver. A potential major factor limiting the efficacy of adenoviral therapies is loss of the main cellular receptor for adenovirus, CAR, in cancer cells. We found that CAR expression is frequently altered in cancer and that a significant number of human tumors lost expression of the receptor. This reduction in expression is, at least in part, mediated by signaling through the Ras/Raf/ERK pathway. Pharmacological inhibition of this pathway increased CAR expression at the cell surface and enhanced cell killing by replication-selective adenoviruses, suggesting that pharmacological receptor restoration could act synergistically with therapeutic adenoviruses.

386

CDC25 phosphatases and checkpoint controls

G. Draetta[1], M. Donzelli[2], M. Squatrito[2], D. Ganoth[3], A. Hershko[3], M. Pagano[4]. [1]*European Institute of Oncology and Pharmacia Oncology, Oncology Research, Milan, Italy;* [2]*European Institute of Oncology, Milan, Italy;* [3]*Technion-Israel Institute of Technology, Haifa, Israel;* [4]*New York University Medical Center, Pathology, New York, USA*

The Cdc25 dual-specificity phosphatases control progression through the eukaryotic cell division cycle by activating cyclin-dependent kinases. Cdc25 A regulates entry into S-phase by dephosphorylating Cdk2, it cooperates with activated oncogenes in inducing transformation and is overexpressed in several human tumors. DNA damage or DNA replication blocks induce phosphorylation of Cdc25 A and its subsequent degradation via the ubiquitin-proteasome pathway. We have investigated the regulation of Cdc25 A in the cell cycle. We found that Cdc25 A degradation during mitotic exit and in early G1 is mediated by the APC/CCdh1 ligase and that a KEN-box motif in the N-terminus of the protein is required for its targeted degradation. Interestingly, the KEN-box mutated protein remains unstable in interphase and upon ionizing radiation exposure. Moreover, SCF inactivation using an interfering Cul1 mutant accumulates and stabilizes Cdc25 A protein. The presence of Cul1 and Skp1 in Cdc25 A immunocomplexes suggests a direct involvement of SCF in Cdc25 A degradation during interphase. We propose that a dual mechanism of regulated degradation allows for fine tuning of Cdc25 A abundance in response to cell environment. These topics will be discussed in the context of the overall approach to cancer drug discovery.

387

Polo-like kinases and mitotic control

D.K. Ferris[1], J.H. Yuan[1], R. Fisher[1], Y. Feng[1], S. Maloid[1], A. Alberts[2]. *NCI, Biological Mechanisms Section Basic Research Laboratory, Frederick, USA; (2) Van Andel Institute, Laboratory of Cell Structure and Signal Integration, Grand Rapids, USA*

Polo-like kinase (Plk1) is an important regulator of several events during mitosis, including, activation of p34cdc2, centrosome maturation, golgi fragmentation and activation of APC and proteasomes. Plk is also a target of a mitotic DNA damage checkpoint. We have examined both the G2 and mitotic DNA damage checkpoints in multiple cell lines. We found that DNA damage introduced during interphase prevented Plk phosphorylation and activation in a caffeine sensitive fashion. In contrast, DNA damage introduced during mitosis in synchronized cells caused Plk activity to be down regulated as a result of dephosphorylation. Mitotic Plk1 dephosphorylation occurs in ATM mutant cells, although these cells have a defective mitotic checkpoint. Evidence that the PI 3 kinase pathway is involved in regulating the Plk1 response to mitotic DNA damage will be presented. Lastly, new data will be shown suggesting a novel role for Plk1 during interphase.

Friday 22 November

PLENARY SESSION 10

Genomic integrity and DNA damaging process

388

NF-kB: a factor that provides a link between stress, inflammation and cancer

M. Karin[1], G. Bonnizi[2], Y. Cao[3], M. Delhase[4], F. Greten[5], Y.L. Hu[6], Z.W. Li[7], C. Makris[8], U. Sentfleben[9]. [1]*UCSD School of Medicine, Laboratory of Gene Regulation and Signal Transduct, La Jolla, USA;* [2]*UCSD School of Medicine, Laboratory of Gene Regulation and Signal Transduct, La Jolla, USA;* [3]*UCSD School of Medicine, Laboratory of Gene Regulation and Signal Transduct, La Jolla, USA;* [4]*UCSD School of Medicine, Laboratory of Gene Regulation and Signal Transduct, La Jolla, USA;* [5]*UCSD School of Medicine, Laboratory of Gene Regulation and Signal Transduct, La Jolla, USA;* [6]*University of Texas, MD Anderson Cancer Center, Smithville, USA;* [7]*UCSD School of Medicine, Laboratory of Gene Regulation and Signal Transduct, La Jolla, USA;* [8]*Merck Frosst, Laval, Canada*

IKK ? A Master Regulator of Innate and Adaptive Immune Respsonses The IkB kinase (IKK) complex is composed of 3 subunits: IKKa, IKKb, and IKKg. IKKa and IKKb, the catalytic subunits, display a high degree of biochemical and structural similarity, both functioning as IkB kinases *in vitro*. The physiological function of the different IKK subunits and the reason for duplication of the catalytic subunits was probed by gene disruption and knockin experiments. At their outset, their experiments demonstrated a critical function for IKKb in activation of NF-kB in response to a large number of proinflammatory stimuli, including TNFa, IL-1, dsRNA, LPS and ISS-DNA. IKKb is also essential for prevention of TNFa induced apoptosis and is indispensable for activation of innate immune responses. All of these IKKb- specific functions depend on IkB phosphorylation and degradation and are mediated through the canonical NF-kB activation pathway. By contrast, the biological functions of IKKa were found to be rather complex. Although IKKa was found not to be required for activation, the canonical NF-kB pathway in response to proinflammatory stimuli, it was found to be essential for skin and bone morphogenesis. The role of IKKa in epidermal differentiation, however, does not depend on its protein kinase activity or on NF-kB. Recently, IKKa was found to have a second function ? being required for activation of a second NF-kB pathway based on the processing of NF-kB2/p100 to p52. This function does depend on the kinase activity of IKKa and seems to be triggered only by select members of the TNF family. This function is required for adaptive immune responses and proper organization and development of lymphoid organs. A third function of IKKa that is also dependent on its kinase activity is in development of the mammary gland. This function is exerted via the canonical NF-kB pathway (i.e. degradation) but is not triggered by standard proinflammatory stimuli. In summary, duplication of the IKK catalytic subunits has enabled the assumption of diverse biological functions that are differentially dependent on IKKa and IKKb.