232 INVITED

Transcriptional regulation of apoptosis by the p53 tumor suppressor protein

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Our laboratory studies the processes by which p53 regulates transcription and apoptosis. We found that the product of the Cellular Apoptosis Susceptibility gene (CAS/hCse1L) associates and co-localizes with p53 in high molecular weight chromatin containing complexes. CAS/hCse1L binds to a subset of p53 target promoters and down-regulation of CAS/hCse1L selectively decreases transcription from those promoters to which it binds and also reduces apoptosis. CAS/hCse1L silencing also affects methylation of select histone H3 residues in the Pig3 promoter. Therefore the ability of CAS/hCse1L to associate with chromatin and with p53 has functional consequences that affect the cellular response to p53.
We compared the apoptotic features of cells undergoing DNA damage-facilitated apoptosis induced by wild-type or mutant p53 with mutated trans-activation domain I (p53Q22/S23). Apoptosis induced by p53(Q22/S23) occurs with relatively slowly and does not activate of caspases 3, 6, 7, 8 and 9. Nevertheless apoptosis induced both by wild-type p53 and p53(Q22/S23) involves caspase-2 activation which is required for release of cytochrome c. Remarkably, p53(Q22/S23) induces the p53 target gene PIDD to the same extent as wild type p53. PIDD, RAIDD and caspase 2 were shown to associate and form a "PIDDosome" that activates caspase 2. We found that siRNA mediated downregulation of each of these 3 genes markedly reduces apoptosis induced by p53(Q22/S23). Thus, the initial stage of DNA damage facilitated p53-mediated apoptosis occurs by a novel caspase-2-dependent mechanism that does not require p53′s full transcriptional regulatory functions but which requires transcriptional induction of PIDD.
Most missense p53 mutations in tumors are within the core DNA binding domain. One region of the core, the L1 loop (residues 112–124) is a mutational "cold spot" in tumors. The p53 L1 loop was subjected to alanine and arginine scanning mutagenesis and select mutants were examined for DNA binding in vitro, and transactivation and cell cycle analysis in vivo. Two mutants displayed exceptionally interesting properties; T123A was markedly more active than wild-type p53 in producing apoptosis, while K120A, although capable of strong binding in vitro and wild-type levels of transactivation and apoptosis when transfected into cells, showed impaired activity when expressed at normal cellular levels. A weaker affinity for DNA in vivo by K120A p53 is the main reason for its defects in transactivation and apoptosis. Overall, the L1 Loop is important in p53′s recognition of specific DNA sequences, target transactivation, and apoptotic signaling.

233 INVITED

Single nucleotides in the p53 pathway

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In the cells of the body the p53 gene and its protein respond to environmental stresses. After an exposure to radiation or a mutagen, the p53 protein is activated and initiates a program of cell death, or apoptosis, eliminating clones of cells that carry mutations and that could develop into cancers. Individuals with somatic or germ line mutations in the p53 gene develop cancers at a very high frequency and at an early age. This is why the p53 gene has been called a tumor suppressor gene. Single nucleotide polymorphisms (SNP) in the p53 pathway have been identified that enhance or reduce the efficiency of the p53 pathway to eliminate potential cancerous cells in the body. A polymorphism in the MDM-2 gene, which regulates p53 protein levels by degrading the p53 protein, has been identified and termed SNP 309. Most individuals have a T-residue at the promoter element of the MDM-2 gene and this produces low levels of the MDM-2 m-RNA and protein. A small percentage of individuals in the population have a G-residue in that location and this produces about four fold more MDM-2 m-RNA and protein, which in turn lowers p53 levels and weakens the p53 apoptotic response to DNA damage. Individuals with the G/G genotype tend to develop cancers more frequently and at a younger age (about 10−12 years earlier) than those with a T/T genotype. The MDM-2 gene is also regulated by the estrogen receptor so that pre-menopausal females with a G/G genotype are at the highest risk for developing some types of cancers at earlier ages. These observations help to explain the genetic basis of gender differences in cancer. In addition this type of information can form the basis for identifying those women at highest risk for taking hormone replacement therapy or identifying those individuals who should be screened for cancers at earlier ages.

234 INVITED

Tumor suppression by p53: inside and outside the cancer cell

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p53 plays a major role in restraining tumorigenesis. Consequently, p53 mutations are found in about 50% of all human tumors. In incipient tumor cells, p53 is crucial for activating cell cycle checkpoints that prevent further neoplastic progression. Data will be presented addressing the role of p53 in prevention of polyploidization via activation of a mitotic apparatus damage checkpoint mediated by the Lats2 tumor suppressor. In response to mitotic apparatus stress, Lats2 translocates from the centrosome to the nucleus, where it binds Mdm2 and induces p53 activity. This sets in motion a positive feedback loop, involving the transactivation of the Lats2 gene by p53, which eventually leads to activation of the "tetraploidy checkpoint" and prevention of polyploidization. Ablation of either p53 or Lats2 has similar consequences on this checkpoint, allowing the emergence of cells with polyploid genomes. A role for Lats2 in cancer is supported by the finding of Lats2 promoter hypermethylation in many tumors. Evidence will also be provided in support of the notion that p53 may serve as a tumor suppressor not only within cancer cells but also in the adjacent stroma. In particular, p53 suppresses the expression and production of the cytokine SDF-1/CXCL12 in cultured stromal fibroblasts. Functional consequences and possible implications for cancer progression will be discussed.

Thursday 9 November 13:00–18:00

ITCC PAEDIATRIC ONCOLOGY SESSION

Paediatric drug development

235 INVITED

Changing EU legislation on medicines for children: a tool for oncology?

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The final European Paediatric Regulation has been voted beginning of June 2006 and is expected to enter into force by end of 2006. It will introduce a dramatic change in the way medicines are developed in and for children. The Regulation's aims are to increase availability of and information on paediatric medicines, and stimulate high quality ethical paediatric research, whilst avoiding unnecessary duplication of paediatric trials.
It will now be mandatory for companies to discuss and agree with a Committee composed of paediatric experts from all Member States a Paediatric Investigation Plan and then its results, or to request a waiver, for any new medicinal product. Depending on the disease and the product, necessary drug development in children would have to be completed before the marketing authorisation in adults or could be delayed until after. A 6-month extension of the patent is the reward for such complete development. At the difference of other specialities medicines used in oncology have been studied through well-established networks, but generally not authorised due to a lack of commercial interest. The major issue for oncology, but not for oncology only, is the difference between adult and paediatric indications. What will be the leeway for the Paediatric Committee to require development in indications that differ in children compared to adults? In oncology the drug's mechanism of action may be more relevant than the indication. The need to go beyond adult indications is not disputed from a scientific perspective, but the legislation is now silent on this aspect and it is likely that legal challenges will define the legal basis. Learning from the US experience, old (off-patent) products devoid of commercial interest have not been forgotten. Additional data protection will be given to new paediatric indications with age-appropriate formulations. Funding of research on old products is also provided for, through the Framework Programmes. In addition, the EMEA will establish a network of existing paediatric research network to facilitate and stimulate research on medicinal products used in children.
Finally, transparency of clinical trial information has been secured by this Regulation with public access to paediatric trial protocols and results. As a conclusion the new Paediatric Regulation is a big opportunity to progress the use of medicinal products in children in their own best interest.