Abstracts: Session III

van der Pluijm, Gabri [49]

Monitoring metastatic behavior of human breast cancer cells in mice with species-specific PCR and bioluminescent reporter imaging

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Breast cancer metastasizes frequently to the skeleton and causes considerable morbidity. Injection of human MDA-MB-231 breast cancer cells into the left heart ventricle of immunodeficient mice produced multiple osteolytic bone lesions and soft tissue metastases. However, micrometastases are not readily detectable and more sensitive methods are required to detect minimal disease states. Here we describe the use of species-specific PCR (ssPCR) and bioluminescent reporter imaging (BRI) for monitoring the tumor metastatic *in vivo*. We used a CCD camera connected to the Argus-20 image processor (C-2400/VIM, Hamamatsu) to monitor bone metastasis by tumor cells transfected with the luciferase reporter gene. MDA-MB-231 cells were stably transfected with a pCMV-plasmid containing the firefly luciferase gene (MDA-231/luc+). After intracardiac inoclation with MDA-231/luc+ cells, the mice were monitored weekly for the development of bone and soft tissue metastases. For this, the mice were anaesthesized, injected with D-luciferin and photon emission was measured. Micrometastases developed predominantly in the hind limbs and the axial skeleton. Distinct photon emission was detected already after 24 days in bone (detection limit ± 10.000 cells). ssPCR revealed significantly increased expression of VEGF-A, -B and PTHrP by the cancer cells in bone metastases when compared to soft tissues metastases suggesting a causal role in the observed preferential skeletal metastasis. In conclusion, BRI and ssPCR can be used for quantitative detection and growth of micrometastases and may become extremely useful to study the pathogenesis of bone metastasis in living animals, to monitor expression of targeted gene vectors and to determine the efficacy of novel therapeutic agents.

Vandesompele, Jo [50]

Integration of suppression subtractive hybridization, laser capture microdissection and microarray analysis for identification of genes implicated in neuroblastoma pathogenesis

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Neuroblastoma is the most frequent extracranial solid tumor in children, showing remarkable clinical and genetic heterogeneity. To make further progress in understanding the genetic basis of neuroblastoma we have applied suppression subtractive hybridization for the identification of differentially expressed transcripts. We analyzed two neuroblastoma cell lines belonging to different genetic subgroups, respectively with and without 1p and 11q deletion and *MYCN* amplification. Northern blot analysis and real-time quantitative polymerase chain reaction with reverse transcription showed differential expression in 75% of the selected genes. We identified both rare and highly abundant differential transcripts. Known and previously unreported genes from the 2p23 and 2p13–14 amplified regions in IMR32 were efficiently selected. From the present set of suppression subtractive hybridization clones, we will select genes relevant to normal neurogenesis through expression analysis of normal fetal neuroblasts isolated by laser capture microdissection (Arcturus, PixCell II). We will select genes implicated in particular subsets of neuroblastoma by microarray analysis (Affymetrix 417) of a large panel of neuroblastoma cell lines and primary tumors.

Vandesompele, Jo [51]

Quantification and normalization of gene expression using SYBR Green I real-time RT-PCR

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We have developed a two-step, real-time, quantitative assay, using the polymerase chain reaction with reverse transcription (RT-PCR) and based on SYBR Green I monitoring of PCR product accumulation, for quantification and normalization of gene expression levels. Because housekeeping gene expression can vary considerably among cell types or experimental conditions, our procedure uses multiple internal control genes for more accurate normalization of expression data, compared with traditional use of only one housekeeping gene. Owing to extensive accumulation of primer-dimers when no template control is used during one-step RT-PCR, we introduced a two-step protocol to eliminate this problem. This study further illustrates the prerequisite of DNase treatment of RNA samples before complementary DNA synthesis. The treatment also results in a significantly facilitated primer design for RT-PCR, as the positions of the primers are no longer important to control for genomic contamination. Real-time quantitative RT-PCR of DNase-treated samples and normalization using multiple internal controls is the method of choice for sensitive, accurate and large-scale measurements of gene expression levels.

Varley, Jenny [52]

Genotype and phenotype in carriers of germline *TP53* mutations

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We collected data on a large cohort of families with features of Li-Fraumeni syndrome (LFS). To date we have identified germline *TP53* mutations in 28 families: 20 of 25 classic LFS and 8 of 20 Li-Fraumeni–like. In addition we have identified germline mutations in 12 other individuals or families with tumors suggestive of LFS. Most mutations are missense or nonsense, but 7 (18%) are splicing mutations. A survey of the literature showed that this is a considerably higher propor-