morigenesis, metastasis and drug resistance as illustrated here by three examples of breast and prostate cancer research: 1) Amplification of the 20q13 chr region was found in breast cancer suggesting the location of a new important oncogene. 2) Distant metastases of breast cancer were often found to be remotely, if at all, clonally related with the primary tumors. Measurements of e.g. prognostic factors from the primary tumors may therefore not reflect the biological properties of metastatic tumor cells. 3) In prostate cancer, amplification of the Xq12 region, the site of the human androgen receptor (AR) gene, emerged in conjunction with the development of resistance to androgen deprivation. AR amplification was found in ≈ 25% of recurrent prostate tumors and apparently facilitated tumor cell growth in low androgen concentrations. These clinical implications of CGH illustrate the power of new genetic screening tools in dissecting the genetic basis of cancer progression.

445 ORAL

RECIPROCAL PATTERNS OF C-ERBB-2 THREONINE AND TYROSINE PHOSPHORYLATION IN HUMAN TUMOUR CELLS

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Overexpression of the c-erbB-2 receptor is implicated in the pathogenesis of human breast cancer, but the functional significance of this phenotype is unclear. We have developed phosphothreonine-specific antibodies recognizing the c-erbB-2 juxtamembrane consensus site for protein kinase C (PKC). Using these antibodies we show that a number of human cancer cell lines exhibit constitutive c-erbB-2 threonine phosphorylation. Unlike nonmalignant fibroblast cell lines, these tumour cells sustain little if any enhancement of receptor threonine phosphorylation following phorbol ester treatment. DNA sequencing reveals no abnormality of juxtamembrane domain structure in any of these cell lines. Hence, PKC may be constitutively activated in some human tumour cells.

446 ORAL

ENHANCED EXPRESSION OF C-MYC, N-MYC, C-HA-RAS 1, C-ERB B-2/NEU, C-FOS AND C-JUN IN HUMAN GERM CELL TUMORS

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The presence of c-myc, N-myc, c-Ha-ras 1, c-erb B-2/neu, c-fos, and c-jun was investigated in biopsy specimens from 20 patients with various types of germ cell tumors. The expression of the oncogenes was demonstrated by in situ hybridization.

C-myc oncogene expression was found in 10/20 of tumors including 2 embryonal carcinomas, in one Leydig cell tumor, in 3 embryonal carcinomas with seminomas, in one immature teratoma with seminoma, in one immature teratoma with choriocarcinoma, in one embryonal carcinoma with immature teratoma, and in one mature teratoma. N-myc was observed in 8/20 of tumors including 2 embryonal carcinomas, in 3 embryonal carcinomas with seminomas, in one embryonal carcinoma with immature teratoma, in one immature teratoma with choriocarcinoma, and one immature teratoma with seminoma. C-Ha-ras 1 oncogene expression was found in 7/20 tumors including 2 embryonal carcinomas, in one mature teratoma, in the benign Leydig cell tumor, in 2 embryonal carcinomas with seminomas, and in one embryonal carcinoma with immature teratoma. Expression of c-erb B-2/neu oncogene could be identified in benign Leydig cell tumor only. C-fos was expressed in 12/20 tumors including in the benign Leydig cell tumor, in 4 pure seminomas, in 3 embryonal carcinomas with seminomas, in 2 immature teratomas, and in one immature teratoma with choriocarcinoma.

C-jun expression was observed in 14/20 tumors, including in 6 pure seminomas, in the benign Leydig cell tumor, in 2 immature teratomas, in one mature teratoma, in 2 embryonal carcinomas with seminomas, in one immature teratoma with choriocarcinoma, and in one embryonal carcinoma with immature teratoma.

The evidence of these oncogenes in human testicular cancer is consistent with the view, that alterations of these oncogenes play a role in the pathogenesis of this tumor type.

447 ORAL

CLASSICAL GENE AMPLIFICATIONS IN HUMAN BREAST CANCER ARE NOT RESPONSIBLE OF DISTANT METASTASES

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Tumour progression is a fundamental feature of biology of cancer. In breast cancer, no genetic events that are critical in the late stages of the tumorigenic process have been identified. To define the relationship between breast cancer progression and gene amplifications, we analysed 62 distant metastases (18 solid metastases and 44 pleural effusions), and 122 primary breast tumours for the three regions most frequently amplified in primary breast carcinomas (protooncogenes *MYC* and *ERBB2* and 11q13 chromosomal region). Surprisingly, *MYC* gene (and also *ERBB2* but at a lower level) was unfrequently amplified in metastases compared with primary breast carcinomas. Furthermore, the solid metastases did not show amplifications for any of these three regions.

These results suggest that protooncogenes *MYC* and *ERBB2* and 11q13 chromosomal region are mainly involved in the genesis of the tumour at its primary site and not in its progression.

448 ORAL

PCR-SSCP A SENSITIVE AND RAPID METHOD TO DETECT MUTATIONS IN THE P53 TUMOR SUPPRESSOR GENE OF PATIENTS WITH ADVANCED COLORECTAL CANCER

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Mutations in the p53 tumor suppressor gene, located on chromosome 17p, are known to be the most frequent genetic alterations found in human cancers. We used the polymerase chain reaction (PCR) followed by the single-strand conformation polymorphism (SSCP) analysis to screen for mutations of the p53 gene in patients of the European Saar-Lor-Lux area with colorectal cancer at various developmental stages. While we detected no mutations in all of 16 early-staged colonic polyp samples, we revealed 7 (13.7%) transition point mutations in exons 5 to 9 of the p53 gene in 51 late-staged colorectal tumours. These results show that the PCR-SSCP analysis technique provides both a sensitive and rapid method for the genetic staging of colorectal samples and confirm previous reports that p53 mutations are usually associated with an advanced development of colorectal cancer characterized by the transition from adenoma to carcinoma.

449 ORAL

BRCA1 GENE MUTATION CARRIER ANALYSIS IN FAMILIAL BREAST CANCER PATIENTS

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Myriad Genetics

BRCA1, the predisposing gene for familial breast and ovarian cancer localized on chromosome 17q21.3 has been recently cloned. The gene comprises 22 coding exons which span 100 kB of genomic sequence. The protein predicted is 1863 aminoacids long. 31 mutations have been identified in constitutional DNA of affected member from various family of which 22 were distinct. Not one of 22 transcribed exons seems to be a preferential target site for mutations. Overall germline BRCA 1 mutations account for 1–2% of all breast cancers and about 3% of ovarian cancers. The lifetime risk of breast and ovarian cancer in BRCA1 mutation carriers is high: the risk of breast cancer is about 50% by age 50 and 70% by age 70. The average risk of ovarian cancer is 40% by age 70. BRCA1 has never been found mutated in sporadic carcinomas, however, loss of heterozigosity in the region of BRCA1 has been observed in 40% of breast and about 60% of ovarian cancers. The lifetime risk to