

Akt3 in these cell lines. This increased activity also correlated with faster proliferation rates and increased survival in the absence of serum. Treatment of the cells with the PI3K inhibitor LY294002 resulted in increased cell death in those cells with high Akt activity. Akt isoform expression was assessed in 53 primary ovarian tumour samples revealing high Akt3 expression in 33% mucinous, 59% serous, and 66.6% endometrioid tumours. High Akt2 expression was observed in 11% mucinous, 18% serous but not in endometrioid tumours. Positive phospho-S473 staining, representing active Akt, correlated with high Akt3 expression.

**Conclusion:** These results suggest that Akt3 may play an important role in ovarian tumourigenesis.

634

POSTER

### The cytochrome p450 (CYP) family 1 at early stages of carcinogenesis.

A. Vaiman. *Institute of Carcinogenesis, N.Blokhin Cancer Rese, Laboratory of Tumor Cell Genetics, Moscow, Russian Federation*

**Background:** CYP1 plays an important role in activation of environmental carcinogens including polycyclic aromatic hydrocarbons, PAH and some drugs. It was suggested that PAH is present in tobacco smoke and participates in tumor development in smokers. There are many studies of CYPs in cancers, however the data on regulation of CYP isoforms at early stages of carcinogenesis are limited. Using two models of cell immortalization and transformation, we studied mRNA expression and activity of CYP1 enzymes as well as the expression of *AHR* and *ARNT* genes that encode the regulators of CYP1 expression.

**Material and methods:** cultivation of cell culture, RNA isolation, RT-PCR, benzo/a/pyrene-hydroxylase activity assay, cell transfection, cell survival assay.

**Result:** In the embryo rat fibroblasts (RF) constitutive level of *CYP 1A1* mRNA was not detectable, whereas *CYP1B1* mRNA was expressed. After cells immortalization with Rauscher virus (F-27/RLV), mRNA level of *CYP 1A1* became high, and *CYP 1B1* level increased in comparison with RF cells. The F-27/RLV cells oxidized benzo/a/pyrene more effectively and were more sensitive to toxic effects of benzo/a/pyrene and 7.12-dimethylbenz/a/anthracene than RF cells. In spontaneously immortalized embryonic rat fibroblasts (Rat1) we found high expression of *CYP1B1* mRNA compared to RF cells. Treatment with TCDD increased *CYP 1B1* mRNA level in both rat cell lines. Unlike RF, Rauscher immortalized cells with relatively high level of CYP1 expression were sensitive to transforming effect of benzo/a/pyrene. In transformed clones levels of *CYP1A1* and *CYP1B1* mRNA were lower compared with F-27/RLV cells. Benzo/a/pyrene - hydroxylase activity decreased in transformed cells. In Rat/ras transformed cells obtained after transfection of *N-ras*<sup>asp12</sup> gene, the constitutive expression of *CYP1B1* mRNA disappeared in comparison with Rat1 cells. The mRNAs of proteins which take part in the regulation of enzymatic induction of CYP1 (*AHR* and *ARNT*) were the same in all cell models studied.

**Conclusion:** Constitutive and inducible levels of *CYP1B1* mRNA increase after immortalization. Transformation of immortalized cells provokes disappearance of *CYP1B1* expression. Since *AHR* and *ARNT* expression are similar in all cells studied, we suggest that other factors besides *AHR* and *ARNT* take part in CYP1 regulation.

## Paediatric oncology

635

POSTER

### Second tumors after treatment for Hodgkin's lymphoma (HL) in children

S. Safonova, A. Malinin, Yu. Punanov. *Cancer Research Institute, Paediatric, St.Petersburg, Russian Federation*

Second malignant tumors (SMT) are one of the most severe complications in children treated for HL. The aim of the study was to investigate the incidence of second tumors in children with HL.

**Materials and methods:** We observed 450 pts. in CR after treatment of HL from 1972 to 1997. The period after treatment was 60-360 months (med.=134.5). The age of pts was from 2 to 18 years (med.=12.5 yrs; M/F=2,6/1). Histological subtypes nodular sclerosing and mixed cellularity were predominance. The III and IV stages of HL were reveal in 70% patients. Practically all children were undergo combined treatment HL,

which were include chemotherapy (MOPP, COPP, ABVD, OPFA, PCVP) and radiotherapy (20-50 Gy).

**Results:** Second tumors were found in 20 pts (4.4%). Malignant tumors were in 12 pts (2.7%), and benign tumors 8 pts (1.8%). Among SMT were: stomach cancer -3, breast cancer -2, thyroid cancer -1, liposarcoma -1, gliosarcoma -1, malignant schwannoma -1, rhabdomyosarcoma -1, acute myeloblastic leukemia -1. SMT were reveal in period from 30 to 340 months to beginning the treatment HL (med. =165 months). Mortality rate for patients with SMT compose 41.7%. Second benign tumors (SBT) were diagnosed in 8 pts during 46-300 months (med.=153). Thyroid adenoma were in 5 pts, breast fibroadenoma 2, papillomatosis of larynx 1. One patient suffered from 2 SBT thyroid adenoma and neurinoma.

**Conclusion:** SMT more often reveal in patients with HL, which treated in age older 10 years (8 pts from 12) and in women (8 pts from 12). All the second solid tumors (SMT and SBT) are localized in zone of radiotherapy with dose 40 Gy. Thyroid and breast tumors are the most frequent in structure of second tumors.

636

POSTER

### Neuroblastoma in adolescents and adults: analysis of a mono-institutional series of 33 consecutive patients.

M. Podda<sup>1</sup>, D. Polastri<sup>1</sup>, M. Massimino<sup>1</sup>, P. Collini<sup>2</sup>, L. Gandola<sup>3</sup>, L. Piva<sup>4</sup>, M. Terenziani<sup>1</sup>, M. Casanova<sup>1</sup>, F. Fossati Bellani<sup>1</sup>, R. Luksch<sup>1</sup>. <sup>1</sup>Istituto Nazionale dei Tumori, Pediatric Oncology, Milano, Italy; <sup>2</sup>Istituto Nazionale dei Tumori, Pathology, Milano, Italy; <sup>3</sup>Istituto Nazionale dei Tumori, Radiotherapy, Milano, Italy; <sup>4</sup>Istituto Nazionale dei Tumori, Surgery, Milano, Italy

**Background:** Neuroblastoma (NBL) is the most common extra-cranial solid tumor in children. More than 95% of patients at diagnosis are younger than 10 years. Adolescents and adults have a grave prognosis, but may have a more indolent course.

**Material and methods:** From 1980 to 2002, 33 patients (stage I= 3, stage II= 6, stage III=8, stage IV=16) with newly diagnosed NBL older than 12 have been admitted at Istituto Nazionale dei Tumori di Milano. Median age was 17 yrs (range 12-69); M/F ratio was 1.2. Symptoms were present and disregarded for many months in the majority of cases: the mean time frame between the onset of symptoms and diagnosis was 15 months. Site of the primary tumor was retroperitoneum in 19 cases, mediastinum in 5, pelvis in 1, cervical in 1, while 6 had an esthesioneuroblastoma; 1 case the primary tumor was unknown. LDH was elevated in 15/33 pts. *Treatment applied:* surgery alone for stage I; post-operative radiotherapy for stage II; stage III and IV received chemotherapy regimens including anthracycline + cyclophosphamide + vincristine ± ifosfamide ± etoposide ± cisplatin. In addition, 10/16 stage IV pts were submitted to sequential hemi-body irradiation as consolidation treatment. Radiotherapy and/or surgery on primary and metastases were decided on individual basis.

**Results:** 20/33 relapsed: 0 stage I, 1 stage II, 4 stage III, 13 stage IV, and 18 relapsed died. The median follow-up is 42 months (range 12-264). EFS and OS probability at 5 years are shown in the table:

	Stage I	Stage II	Stage III	Stage IV
EFS	1	0.67	0.40	0
OS	1	0.83	0.56	0.12

In this series a consistent number of late relapse/progression were observed: time to progression/relapse ranged from 3 to 58 months and the time from relapse to death from 2 to 75 months. In univariate analysis, together with the stage, the only statistically significant prognostic factor is LDH level at diagnosis: an elevated LDH negatively predicted the outcome (5 yrs OS: normal 54%, pathological 0; p 0.0089).

**Conclusions:** Localized NBL (stage I and II) in adolescents and adults have the same good prognosis of children. For pts with locally advanced and metastatic disease, late events were frequently observed, thus suggesting a lower biological aggressiveness of the disease in this subset. Nevertheless, the prognosis for these patients is dismal.

637

POSTER

### Evidence for a redox mechanism of action of prednisolone in childhood acute lymphoblastic leukaemia through the identification of the novel gene CGI-31.

A. Burke, P. Kearns, N. Goulden. *University of Bristol, Pathology and Microbiology, Bristol, United Kingdom*

Glucocorticoids are the most important drugs used in the treatment of acute lymphoblastic leukaemia and poor response to these drugs during