REGISTRATION OF TUMOURS WITH MORPHOLOGICAL HETEROGENICITY: AGE-DISTRIBUTION OF THYROID CANCER IN DENMARK 1978-85.
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Registration of morphological heterogene tumours creates technical problems in the data collection as well as in the analysis. In the Danish Cancer Registry tumours have been coded according to the ICD-O since 1978. This classification includes a few combination codes, but most of the combined morphologies are coded with the higher number of the components. One of the combination codes is "Papillary and follicular adenocarcinoma" (M-8340/3). We have shown earlier that papillary carcinoma and follicular carcinoma of the thyroid have different agedistribution of the mixed type resembled the one or the other. In the Registry, 198 thyroid cancers were coded as follicular, 275 as papillary and 52 as mixed papillary and follicular. A test on agedistribution of the 3 subtypes showed a highly significant difference between follicular and mixed type ($p < 0.003$), whereas the resemblance between papillary and mixed type was so strong that a test for difference between the two distributions produced $p = 0.97$. It is concluded that in this example of morphological heterogenicity, the agedistribution and thus maybe some of the etiological factors could be assigned to one of the components.

HETEROGENEITY OF ESTROGEN RECEPTOR CONTENT IN PRIMARY BREAST CANCERS AND THEIR METASTASES.
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Immunohistochemical antibody techniques for detection of estrogen receptors (ER) were applied to formalin fixed, paraffin embedded sections from 62 primary breast cancers, metastases of the regional lymph nodes (29 cases), the bone marrow (43 cases), and the liver (20 cases). 40% of the primary tumors and 31% of the regional lymph node metastases were ER positive; less than 20% of liver and bone metastases were ER positive. The ER status of regional lymph node metastases was concordant with that of the primary tumor in 90% of the cases. The concordance rate was 75% for liver metastases and 58% for bone metastases. Patients with ER positive tumors had recurrence more often in bone; ER negative tumors recurred more often in the liver. The survival after recurrence (SAR) was related to the ER status of both the primary tumor and the lymph node metastases. The SAR was not associated with the ER status of bone marrow carcinosis or liver metastases. Regression analyses showed that ER status of the primary tumor was the most important independent prognostic factor when compared to other clinical, pathoanatomical and biochemical features. The study supports the hypothesis that tumor cell clones with different ER content are selected and adapted to grow in various anatomical sites.

SUBOPTIMAL CHEMOTHERAPY MAY BE BENEFICIAL IN THE TREATMENT OF HETEROGENEOUS TUMORS
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In the nude mouse model, it was shown that when a mixed tumor of human small cell carcinoma of the lung consists of a dominating subpopulation (592) sensitive to BCNU, and a dominated and silent (undetectable) subpopulation (NYH) which is resistant to BCNU, then treatment will kill the dominating cells only, resulting in a primary tumor response followed by early relapse of the resistant cells (Eur.J.Cancer Clin.Oncol.24:1550,1988). In this study, mixed tumors of the sensitive (592) and resistant (NYH) cells in nude mice were given suboptimal BCNU treatment, resulting in a minor reduction in tumor size without complete eradication of the sensitive and dominating subpopulation. At regrowth of the tumors, still only the sensitive 592 tumor could be detected by flow cytometric DNA analysis by which the two cell lines were distinguishable. Not until a subsequent optimal BCNU treatment had caused complete disappearance of the 592 cells, relapse of the resistant subpopulation (NYH) took place. Thus, it is concluded that suboptimal chemotherapy was beneficial in terms of temporary growth control of this tumor.

THE HETEROGENEITY OF SMALL CELL CARCINOMA OF THE LUNG (SCCL) IN SENSITIVITY TO CYTOSTATIC DRUGS REFLECTED IN A PANEL OF CELL LINES.
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SCCL has been shown to be heterogeneous, and treatment failure is caused by selection and overgrowth of resistant subpopulations. A large number of tumor cell lines have been established from patients with SCCL. If these cell lines reflect the heterogeneity in the parent tumor the patterns in sensitivity to different cytostatic drugs may reflect the range of sensitivities in the disease and enable selection of drugs and drug combinations for future treatment protocols. Using in vitro sensitivity assessments we have shown that SCCL cell lines established from treated and untreated patients vary in sensitivity to adriamycin, daunorubicin and mitoxantrone with a factor of five. The sensitivity to

VP-16, VM-26 varies with a factor of two, cisplatin, carboplatin, BCNU, and TCNU with a factor of eight. In the comparison of patterns in sensitivity, the cell lines most sensitive to epipodophyllotoxines and anthracyclines proved least sensitive to platin and nitrosourea and vice versa. By continuous exposure to daunorubicin a five-fold resistant cell line was obtained. Compared to the parental cell line, the same collateral sensitivity was obtained on this cell line, as is was cross-resistant to adriamycin and VP-16 and expressed an increased sensitivity to cisplatin and BCNU. From these results it is tempting to explain the clinical synergy of platin and epipodophyllotoxines as a cytotoxic action on different subclones within the tumor.

HETEROGENEIC EXPRESSION OF BLOOD GROUP A AND H ISOANTIGENS IN BLADDER TUMOURS: ASSOCIATION WITH MEAN NUCLEAR VOLUME.
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To elucidate the well known heterogeneity in the ability to express blood group ABO isoantigens in transitional cell carcinomas, a stereological estimate of the mean nuclear volume in areas expressing blood group antigen was compared to the estimate from areas of identical pathological grade where the antigen expression was deleted. Mean nuclear volume were estimated from antigen positive and antigen negative areas in sections from 21 blood group 0 and 20 blood group A individuals using an indirect peroxidase method with monoclonal anti-H and anti-A antibodies. The mean nuclear volume increased as expected with increasing pathologic grade. In blood group 0 individuals the mean nuclear volume was 241 μm^3 in positive areas and 338 μm^3 in negative areas ($2p<0.0005$) of identical pathologic grade. In A individuals the mean nuclear volume was 217 μm^3 in positive areas, and 351 μm^3 in negative areas ($2p<0.0025$). The results indicate a complex biological nuclear mecanism associated with the cellular ability to express blood group antigens.

HETEROGENEITY OF HIGH-ENERGY PHOSPHATE COMPOUNDS IN TWO SCLC XENOGRAFTS BEFORE AND AFTER X-IRRADIATION.
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The in vivo levels of ATP and inorganic phosphate (Pi) were measured in two sublines derived from a single human small cell lung cancer (SCLC). The tumors were serially grown in nude mice. The tumor line CPH SCC 54A is more radiosensitive than 54B in spite of similar growth characteristics. The metabolites were measured by in vivo 31-P-MR-spectroscopy and biochemical analysis of extracts of freeze-clamped tumors. During untreated growth a slow decline in ATP:Pi ratio was seen in both tumors. In 54A the ATP:Pi was significantly higher than in 54B. Irradiation of the tumors with 20 Gy induced an immediate decrease in ATP:Pi with a nadir at 6-12 hours followed by a gradual increase to pretreat- ment levels within 72 hours. The decrease was faster in 54A with a $T_{\frac{1}{2}}$ of 2.5 hours against 6 hours in 54B. This study demonstrated a difference in energy metabolism as well as in the metabolic response to irradiation in two SCLC sublines from the same original tumor.

CLONAL HETEROGENEITY DEMONSTRATED BY FLOW CYTOMETRY AND CHROMOSOME ANALYSIS AS A FACTOR IN THE DEVELOPMENT AND PROGRESSION OF COLORECTAL CANCER.
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The theory of clonal evolution of tumours is mainly based upon cytogenetic analysis of the progression of hematologic malignancies, whereas similar studies on solid tumours are few. Ploidy analysis of 51 colorectal adenomas by flow cytometry (FCM) showed the occurrence of smaller or larger DNA-aneuploid cell populations in 17, with a clear correlation to adenomas with severe dysplasia. Chromosome counting on metaphase spreads from 8 adenomas with moderate dysplasia and 2 with severe dysplasia revealed modal values in the range of 45-50, but 18% of the metaphase counts were scattered in the range 51-110. None of these adenomas showed corresponding aneuploid peaks in the DNA histograms. This indicates that benign adenomas continuously develop a number of aberrant methaphases, which do not form quantitatively significant subpopulations, discernible by FCM. However, selection of one of these may start an aneuploid, possibly more dysplastic clone. FCM of multiple biopsies from 120 colorectal carcinomas showed that 71 (59%) had only one cell population (either diploid or aneuploid). Forty-nine (41%) had 2 or more cell populations with different DNA ploidy either mixed in the tumour or dominating distinct areas. This frequently seen heterogeneity was significantly correlated to a bad survival, but also to advanced tumour stage. Diploidy versus aneuploidy did not give any prognostic information. In a multivariate regression analysis tumour stage was the dominant prognostic factor, with insignificant additional information from DNA heterogeneity.