BIOLOGY OF SARCOMAS

Organizers: Beverly Emanuel, Joseph Madri and Richard Womer

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Poster Sessions

Molecular Cytogenetics-I

CL 001 Approach to a Sarcoma-Associated Chromosomal Translocation: Breakpoint Cloning in the Absence of a Candidate Gene. Beverly S. Emanuel[1], Marcia Budarf[1], Heather McDermid[2], and Jaclyn Biegel[1]. Division of Human Genetics and Molecular Biology at the Children's Hospital of Philadelphia[1] and University of Edmonton, Canada[2].

Numerous non-random translocations have been associated with a variety of neoplastic diseases. At the present time it is generally believed that these translocations produce the cellular genetic changes, such as the alterred or aberrant gene expression or function, etiologic for tumorigenesis. Thus, the t(9;22) of CML and ALL results in the juxtaposition of sequences of the ABL oncogene with BCR sequences, causing an alteration in tyrosine kinase activity for ABL. Likewise, the t(8;14), t(8;22), and t(2;8) of Burkitt's Lymphoma results in deregulation of the MYC oncogene presumably as the result of juxtaposition with immunoglobulin sequences in a B-cell. These and several other examples of non-random translocations, primarily in the hematopoietic neoplasms, have yielded to molecular cloning strategies. In each case, a candidate gene at the translocation junction provided the starting point for attempts at such cloning efforts.

Several sarcomas demonstrate non-random chromosomal translocations. These include the t(11;22) of Ewing's Sarcoma, the t(X;18) of Synovial Sarcoma, the t(2;13) of alveolar rhabdomyosarcoma and several others to be disscussed at this meeting. These chromosomal rearrangements appear to be of diagnostic value and, hence, perhaps pathognomonic. We have been interested in the t(11;22) of Ewing's Sarcoma/Peripheral Neuroepithelioma (ES/NE). Despite extensive mapping efforts, no obvious candidate gene has emerged to facilitate translocation breakpoint cloning. Thus, as a first approach to this cloning, we have isolated somatic cell hybrids for chromosomes 11 and 22, including Ewing's Sarcoma related hybrids with the approriate derivative chromosomes. We have begun to delineate a long range restriction map for 22q11 in an attempt to identify the translocation breakpoint. Our studies represent a prototypic approach to translocation breakpoint cloning in the absence of a candidate gene. The status of our map and our progress toward the Ewing's Sarcoma breakpoint cloning efforts will be presented.

CL 002 WHY DOES THE TRANSFORMATION OF CELLS FROM VARIOUS TISSUES REQUIRE INACTIVATION OF DIFFERENT RECESSIVE ONCOGENES, Stephen H. Friend, MGH Cancer Center, Charlestown, MA, The Childrens Hospital and Dana Farber Cancer Institute, Boston, MA 02115. Emerging evidence conclusively shows that tumor formation requires more than just the activation of dominant oncogenes. There is another class of genes termed recessive oncogenes that are also frequently altered. Unlike the dominant oncogenes, the recessive oncogenes normally function to limit cell growth. It is therefore their inactivation that is tumorigenic. The first recessive oncogene to be isolated was the retinoblastoma gene. It is so called because of its alteration in retinal tumors. It is in fact inactivated in many other tumors. We have found it inactivated in osteosarcomas, soft tissue sarcomas, small cell lung carcinomas and a select group of other tumors. The osteosarcomas have further necessary changes. We have studied these changes in several ways including re-expression of the retinoblastoma gene and other recessive oncogenes. The result is an indication of multiple coordinated growth inhibiting pathways that normally maintain proper cellular growth in a tissue specific manner.

CL 003 **CONSISTENT CYTOGENETIC CHANGES IN SARCOMAS**, Felix Mitelman, Department of Clinical Genetics, Lund University Hospital, S-221 85 Lund, Sweden

In spite of recent technical improvements in cancer cytogenetics, very little information is still available on the cytogenetic abnormalities that characterize malignant mesenchymal tumors. Sufficient data for an informed discussion of tumor karyology exist for only five tumor types: liposarcoma, synovial sarcoma, rhabdomyosarcoma, Ewing's sarcoma, and malignant fibrous histiocytoma.

An apparently highly specific chromosomal rearrangement, t(12;16)(q13;p11), characterizes myxoid liposarcomas. Chromosome abnormalities have so far been reported in less than 30 liposarcomas, but no systematic involvement of chromosomes 12 and 16 has been identified in the few malignant lipogenic tumors of other histologies.

The translocation t(X;18)(p11;q11) has been found in all cases of synovial sarcomas so far studied. Since less than 20 tumors have been investigated, it is too early to know both how frequent and how specific this rearrangement will prove to be. It is noteworthy that t(X;18) is the first cancer-associated rearrangement to involve one of the sex chromosomes.

The reciprocal translocation t(2;13)(q35-37;q14) has been described in more than 40 rhabdomyosarcomas. Although the majority of these tumors were of the alveolar type, the translocation has also been found in cases classified as undifferentiated and embryonal. Since the t(2;13) has so far not been reported in any other tumor type, it may well turn out to be pathognomonic for rhabdomyosarcomas.

A characteristic chromosomal abnormality, t(11;22)(q24;q12), is a consistent finding in Ewing's sarcoma. An apparently identical reciprocal translocation has also been described in neuroepithelioma and in the Askin tumor, both of which may consequently be closely pathogenetically related to Ewing's sarcoma.

The breakpoint distribution of the chromosomal rearrangements identified in about 30 malignant fibrous histiocytomas is clearly nonrandom, with preferential involvement of bands 1q11, 1p36, 3p12, 11p11, and 19p13. Of particular interest may be the observation that tumors with 19p13 abnormalities seem to have a pronounced tendency to recur locally.

Two principal lessons may be learned from the acquired karyotypic anomalies of tumor cells. Firstly, they reveal the fundamental biological mechanisms that are disrupted when normal cells become neoplastic; a basic research interest of almost unlimited future potential. Secondly, and of practical importance already at this stage, the cytogenetic aberrations are parameters of direct clinical relevance.

Gene Expression, Alteration, and Amplification-I

CL 004 MOLECULAR STUDIES OF POORLY DIFFERENTIATED CHILDHOOD SOLID TUMORS, O.M. El-Badry, M. Cooper and M.A. Israel, Pediatric Branch, NCI/NIH, Bethesda, MD 20892

Although an important goal of clinical oncology is to group together malignancies that can be expected to respond similarly to available modalities of antineoplastic therapy, few pathologic classification schema are successful in this regard. We therefore sought to determine the relation of poorly differentiated sarcomas that are frequently resistant to therapy to other childhood tumors that are thought to be more responsive. In the course of these studies, we found a. number of similarities between Ewing's sarcoma and tumors of the peripheral nervous system, raising the possibility that these tumors might share some common biologic features. Of particular interest was the observation that Ewing's sarcoma cells utilize some of the same growth-stimulatory pathways important for the proliferation of such neuronal tumors. We found that, like some neuronal tumors of childhood, Ewing's sarcoma cells can be grown routinely in mitogen-free media, although their proliferation can sometimes be further stimulated by supraphysiologic doses of insulin. Because such cell lines can express mRNA encoding IGF-I but not IGF-II, and also express the type I IGF receptor, we believe it possible that these cells grow in culture through an autocrine mechanism mediated by IGF-I. Interestingly, it has not yet been possible to demonstrate IGF-I in medium conditioned by such tumor cell lines; and alpha-IR3, an antibody that recognizes the type I IGF receptor, does not inhibit growth. Our finding that other tumors of the peripheral nervous system grow in response to mitogens that are produced physiologically in embryonic cells corresponding to the immature cell type in which these tumors arise, raises the possibility that Ewing's sarcoma arises in cells that expressed IGF-I physiologically during development. If the growth-regulatory mechanisms by which specific tumors grow have any impact at all on the manner in which they respond to currently available modalities of antineoplastic therapy, or if these growth regulatory pathways mark some biological feature of malignancy that is of importance in determining their responsiveness, it is possible that apparently diverse tumors may be sensitive to similar types of therapeutic intervention.

Gene Expression, Alteration, and Amplification-II

CL 005 MOLECULAR BIOLOGY AND CLINICAL RELEVANCE OF THE MULTIDRUG TRANSPORTER, Michael M. Gottesman, Mark Willingham, Lori Goldstein, and Ira Pastan, National Institutes of Health, National Cancer Institute, Laboratory of Cell Biology and Laboratory of Molecular Biology, Bethesda, MD 20892

Drug resistance, either intrinsic or acquired, is the major impediment to successful chemotherapy of human cancer. One mechanism of resistance to multiple natural product hydrophobic drugs results from expression of the *MDR*1 gene, whose product, P-glycoprotein, is an energy-dependent efflux pump. This multidrug transporter is expressed in transporting epithelia in the liver, kidney, and intestine, as well as in specialized capillary endothelial cells of the brain and testis, suggesting that it is responsible for transport of exogenous, and perhaps endogenous toxic metabolites in normal tissues. Transgenic mice which express the human *MDR*1 gene in their bone marrow become resistant to the myelosuppressive effects of daunomycin, indicating that *in vivo* expression of the *MDR*1 gene is sufficient to confer drug-resistance. Many human cancers express elevated levels of the *MDR*1 gene in a pattern which suggests an important role for the multidrug transporter in clinical drug-resistance: (1) Expression of the *MDR*1 gene is common in multidrug-resistant human cancers, including adenocarcinomas of the kidney, liver, colon, pancreas, and carcinoid tumors, suggesting that the multidrug transporter may contribute to the intrinsic resistance of these cancers; (2) Elevated *MDR*1 RNA levels are also occasionally seen in certain leukemias and lymphomas prior to therapy, and preliminary data suggest that failure of therapeutic response in some leukemias may be related to these elevated levels; and (3) Some tumors, such as childhood neuroblastomas, leukemias, lymphomas, and ovarian cancer, show elevated *MDR*1 gene expression after relapse from chemotherapy, suggesting that the *MDR*1 gene is involved in the acquisition of multidrug-resistance.

Growth Factors and Tumor/Stroma Interactions-I

CL 006 ENDOTHELIAL PDGF B/c-sis EXPRESSION: HETEROGENEITY OF TRANSCRIPTS AND TRANSCRIPTIONAL CONTROL, Thomas O. Daniel, Zhou Fen, and Xinbo Cheng, Departments of Medicine and Cell Biology, Vanderbilt University, Nashville, TN 37232.

Through interaction with PDGF receptors on responsive cells, excessive expression of PDGF activity transforms cultured cells, and is implicated in pathogenesis of osteosarcomas, fibrosarcomas, and glioblastomas. Endothelial cell-derived PDGF activity may provide proliferative stimuli to stromal and tumor cells, alike. We have characterized mechanisms regulating expression of PDGF B/c-sis transcripts by human renal microvascular endothelial cells (HRMEC), as a model system, and have identified structural and functional features of a second PDGF B transcript expressed in HRMEC.

PDGF B/c-sis mRNA levels are induced 5-10 fold over basal constitutive expression by exposure of HRMEC to transforming growth factor β or thrombin. In contrast, agents which increase cellular cAMP levels, including forskolin and β adrenergic agents, dominantly suppress PDGF B mRNA expression, even in the presence of inducing agents. Both induction and suppression of PDGF B mRNA levels are mediated through changes in PDGF B/c-sis gene transcription rates, demonstrated by transcription run on assays and actinomycin D decay studies that show these agents do not alter on the PDGF B mRNA half life of 70-90 min (1). Changes in mRNA levels are paralleled by changes in conditioned media PDGF activity. The transcription repression effect of cAMP is sensitive to inhibitors of cAMP dependent protein kinase, implying a role for phosphorylation in transcription regulation.

Protein synthesis inhibition, by cycloheximide, anisomycin or puromycin, promotes appearance of a second PDGF B transcript, approximately 1kb shorter than the native transcript. The shorter PDGF B mRNA lacks 5' untranslated sequences from the 1st exon, excluding three AUG's upstream of the translation initiation site, and sequences previously shown to retard translation of this mRNA. In the presence of cycloheximide, the shortened transcript is 7-10 fold more stable than the full length transcript. The role of this transcript in PDGF B/c-sis mRNA metabolism and protein synthesis will be discussed.

Daniel TO and Zhou Fen (1988). Distinct pathways mediate regulation of PDGF B/c-sis transcription. J Biol Chem 263:19815-19820.

CL 007 TRANSFORMING GROWTH FACTOR-BETA AND REGULATION OF CONNECTIVE TISSUE CELL BEHAVIOR, Kathleen C. Flanders, Anita B. Roberts, and Michael B. Sporn, Laboratory of Chemoprevention, National Institutes of Health, Bethesda, MD 20892

Transforming growth factor-ßs (TGF-ßs) are multifunctional regulators of cell growth and differentiation. Five distinct, yet highly homologous, TGF-ßs have been identified. In most in vitro assays these TGF-ßs behave similarly, although some differences in bioactivity are beginning to be found. Furthermore, differences in the in vivo localization of these isoforms have also been observed as determined by immunohistochemical staining of tissue sections using antibodies specific for each TGF-ß. For example, in the mouse embryo, TGF-ßs 1, 2, and 3 are coexpressed in bone and cartilage, while TGF-ßs 2 and 3 are preferentially localized to the central and peripheral nervous systems. These results suggest that each TGF-ß isoform may have specialized functions, in addition to common biological effects induced by all TGF-ßs.

In the mouse embryo, immunohistochemical studies show that TGF-ßs are strongly expressed in tissues derived from mesenchyme. TGF-ßs also have profound effects on the growth and differentiation of mesenchymal cells. In vitro, TGF-ß is mitogenic for fibroblasts and osteoblasts, inhibits differentiation of adipocytes and myocytes, and has bifunctional effects on growth and differentiation of chondrocytes. TGF-ß also induces the expression of many extracellular matrix proteins and their receptors in cultured fibroblasts, fibrosarcomas, osteoblasts, osteosarcomas, chondrocytes, preadipocytes and myoblasts. In addition, administration of TGF-ß induces angiogenesis, fibrosis, and bone formation in vivo. The localization of TGF-ßs to mesenchymal cells in vivo, along with the effects of TGF-ß on growth and differentiation of these cells in vitro, suggest that TGF-ß may be one of the factors involved in the pathogenesis of sarcomas. Mechanisms may include not only overexpression of TGF-ß, which has been observed for many cells, but also altered response patterns in terms of both synthesis of and interaction with matrix proteins.

CL 008 INTERACTIONS BETWEEN TUMOR CELLS, VASCULAR CELLS AND THE EXTRACELLULAR MATRIX, Joseph A. Madri, Yale University School of Medicine, New Haven, CT 06510 Tumor cells are in intimate contact with the existing surrounding and newly-synthesized extracellular matrix. In addition, in order for tumors to achieve a large size tumor cells must elicit an angiogenic response and in order for tumor cells to metastasize there must be a close association between tumor cells and local vascular endothelial cells. Using a transplantable pancreatic carcinoma we have noted dramatic matrix modulation of tumor cell polarity and multicellular organization in vivo. Namely, although tumor cell parenchyma was devoid of basement membrane components, when in contact with or in close apposition to host vascular adventitia, tumor cells were noted to deposit a basal lamina and polarize. Furthermore, when dispersed tumor cells were plated on interstitial or basement membrane matrices the cells remained unpolarized and disorganized on the interstitial matrix but attached polarized and formed junctional complexes when plated on the basement membrane. In addition to responding to existing matrix tumor cells are also capable of matrix biosynthesis and eliciting new matrix production in surrounding host mesenchymal cells. This new matrix synthesis and deposition can, in part, modulate tumor cell behavior (including differentiation state, rate and extent of local spread and metastatic potential). Tumor cells also interrogate with local microsvascular endothelial cells in a complex fashion. Soluble factors produced and secreted by tumor cells are angiogenic and elicit a neovascular response. The extent and duration of this angiogenic response depends upon the soluble factors present and the composition and organization of the local extracellular matrix.

Growth Factors and Tumor/Stroma Interactions-II

CL 009 DOMINANT SUPPRESSORS OF PDGF GENE EXPRESSION: A NEW TACTIC FOR DEVELOPING ANIMAL DISEASE MODELS. Prescott Deininger*, Mark Mercola, Julie Porter, and Charles D. Stiles, Division of Cellular and Molecular Biology, Dana-Farber Cancer Institute, Boston, MA 02115. The function of any hormone in vivo is best displayed through the physiology of a disease state wherein that hormone (or its receptor) is not expressed. The physiology of diabetes melitis, for example, displays the various functions of insulin by default. With one exception (that being IGF-1), no systemic disease has ever been linked to underproduction of a growth factor or its receptor. We would like to examine the physiology of animals which do not produce, or alternatively, cannot respond to growth factors. We are exploring the value of dominant suppressor genes and we are using platelet-derived growth factor (PDGF) as a test vehicle. We have constructed mutations within a cDNA clone or wild type mouse PDGF A. The mutated proteins retain the ability to form covalently linked dimers with wild type PDGF A subunits; however, the resulting heterodimers are biologically inactive. The dominant suppressor activity of mutated PDGF A chains is readily displayed in cos cell co-transfection experiments. When mutated PDGF A peptides are overexpressed as little as 7-fold (relative to wild type PDGF A), the production of biologically active PDGF A homodimer is decreased by 90% relative to controls. The dominant suppressor mutations can exert their effect across phylogenetic boundaries. For example, the mutations which we constructed in mouse PDGF A cDNA will suppress expression of the Xenopus PDGF A gene in cos cell cultures.

Other growth factors which, like PDGF, function as covalently linked dimers could be studied by this approach. Dominant negative mutations would be easier to introduce into transgenic animals than recessive homologous recombination defects. Dominant negative mutations may also function where anti-sense tactics have proved inadequate, such as in fertilized frog eggs and early embryos.

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Future Directions in Diagnosis, Prognosis and Treatment

CL 010 GROWTH-RELATED MOLECULES IN SARCOMAS & OTHER SOFT TISSUE TUMORS. John J. Brooks, Patricia Perosio, Thomas S Frank, Swen A Swanson, Cynthia Palman, Daniel Bowen-Pope. Department of Pathology and Laboratory Medicine, University of Pennsylvania Medical School, Philadelphia, PA 19104 & University of Washington, Seattle, WA.

We have investigated a variety of growth factors (1,2), growth factor receptors (1,2), oncogenes and oncoproteins, and proliferation markers in sarcomas. Both benign(B) and malignant(M) lesions expressed a variety of factors and receptors (EGF, EGF-R, NGF, NGF-R, PDGF). Expression of single growth factors (90% M vs 55% B, $p<.005$), multiple factors (44% M vs 24% B, $p<.005$), single receptors (73% M vs 31% B, $p<.001$) and multiple receptors (35% M vs 3% B, $p<.005$) was significantly greater in malignant tumors. Factor/receptor co-expression was significantly more common in sarcomas (63% M vs 17% B, $p<.005$). We noted support for an autocrine growth mechanism in both benign lesions and sarcomas (1). Unlike other growth factors & receptors, NGF-R was detectable in paraffin section and has some limited diagnostic utility (2). In a separate study, PDGF-R expression (monoclonal antibody to ß-subunit PR7 212) was observed in fibroblasts, smooth muscle, and endothelial cells. Many tumors were positive including 2/2 malignant fibrous histiocytoma (MFH), 5/6 leiomyoma, 8/9 leiomyosarcoma, 4/4 liposarcoma, 6/7 synovial sarcoma, and 2/2 angiosarcoma. Fibromatosis, reactive fibroblasts, and vascular components were also reactive. PDGF-R was not necessarily related to the presence of PDGF. We concluded that PDGF-R expression can be identified in a wide variety of mesenchymal lesions independent of histologic type and postulate that its presence may be important in the mechanism of growth of these tumors.

Immunohistochemically, sarcomas lacked the c-erb-B-2 oncoprotein; c-myc oncoprotein was noted in essentially all soft tissue lesions. In 7 MFH, m-RNAs for c-sis and TGF-ß were not identified by Northern blots; thus, MFH seems different from normal fibroblasts -i.e., not driven by PDGF (the product of c-sis, which in turn is normally stimulated by TGF-ß).

The proliferation marker Ki-67 antibody showed an overall correlation with lesional grade. Reactive/benign lesions had little immunoreactivity (low score); many malignant tumors (particularly high grade) had a high Ki-67 score. Importantly, there were definite differences possibly related to prognosis among the 34 high grade lesions; 26% had a low, 18% an intermediate, and 56% a high score. Additional work is in progress to determine its prognostic importance. Further, Ki-67 staining did not correlate well with DNA flow S-phase data and seemed to be an independent factor.

1) Lab Invest 60: 245-253, 1989; 2) Am J Pathol 132: 152-160, 1988.

CL 011 CLINICAL IMPLICATIONS OF PLOIDY AND OTHER CYTOGENETIC ABNORMALITIES FOUND IN CHILDHOOD RHABDOMYOSARCOMA. A. Thomas Look, David N. Shapiro, David M. Parham, Edwin C. Douglass, Bruce L. Webber, William A. Newton Jr., and Harold M. Maurer. From the Departments of Hematology-Oncology and Pathology and Laboratory Medicine, St. Jude Children's Research Hospital, Memphis, TN; Department of Pathology, Baptist Memorial Hospital, Memphis, TN; Department of Laboratory Medicine, Children's Hospital, Columbus OH; and the Department of Pediatrics, Medical College of Virginia, Richmond, VA.

Analysis of clinical and histopathologic features is often inadequate to predict relapse or survival of individual patients with rhabdomyosarcoma. We therefore studied the cellular DNA content (ploidy) of rhabdomyosarcoma cells as it relates to histology and response to therapy in 37 patients with unresectable tumors (Clinical Groups III and IV). Using flow cytometric techniques, we found that about one-third of the patients had diploid tumor stem lines, regardless of histologic type. In the group with abnormal ploidy, hyperdiploidy (1.10 and 1.80 times the DNA in normal diploid cells) was exclusively associated with embryonal histology ($P = 0.001$). By contrast, a near-tetraploid DNA content (1.80 and 2.60 times that of normal cells) was strongly associated with the alveolar histologic subtype ($P = 0.001$). Thus, abnormal ploidy in these histologic subtypes appears to arise by different mechanisms.

Another cytogenetic abnormality closely associated with histologic subtype of rhabdomyosarcoma is the t(2;13)(q35;q14), a specific chromosomal abnormality identified in tumor cells from about one-half of children with disseminated alveolar tumors. The t(2;13) was found in both diploid and tetraploid alveolar tumors, and the near-tetraploid stem lines apparently arise from diploid stem lines by endoreduplication, because derivative chromosomes produced by the t(2;13) are duplicated in the near-tetraploid cells. Tumors with the t(2;13) often have extensive metastasis to bone marrow; 4 of the 14 cases we have identified were referred initially for suspected acute leukemia.

Tumor cell ploidy had a significant impact on survival, with hyperdiploidy conferring the best prognosis and diploidy the worse ($P=0.0001$). None of the 11 patients with diploid tumors survived for more than 18 months. Tumor cell ploidy was the best predictor of treatment outcome in patients with either embryonal ($p<0.001$; relative risk, 25.5) or alveolar ($P=0.073$; relative risk 7.1) rhabdomyosarcoma, and contributed significantly after adjustment for disease stage or anatomic site. Patients with unresectable diploid rhabdomyosarcoma have an unacceptably high risk of treatment failure, justifying new therapeutic approaches for this distinct subgroup. Thus, the ultimate goal of clinical staging, the identification of individual patients at high or low risk of treatment failure, will be substantially improved in rhabdomyosarcoma by systematic studies of tumor cell ploidy, as well as cytogenetic and molecular abnormalities.

CL 012 DIAGNOSIS OF SARCOMAS: CURRENT STATUS AND FUTURE DIRECTIONS. Juan Rosai, M.D., Dept. of Pathology, Yale University School of Medicine, New Haven, CT 06510

The pathologic diagnosis of sarcoma has been traditionally based on the light microscopic examination of hematoxylin-eosin stained sections of formalin-fixed, paraffin-embedded material. The three crucial determinations that the pathologist has to make on the basis of this material are the confirmation of the presence of a mesenchymal malignancy, the determination of the line of differentiation ("cell type"), and an estimation of the degree of malignancy. Special techniques that have been employed over the years in an effort to increase the accuracy of this determination and to provide better and more precise means of characterization include: (1) Conventional special stains: periodic acid Schiff (PAS), reticulin, trichromes, PTAH, etc. Their main purpose has been to further delineate the cell type; the contribution they have made has been limited, largely because of their small degree of specificity; (2) Tissue culture: this technique has also found very limited application, and is currently used in only selected instances; (3) Electron microscopy: this technique is also concerned almost exclusively on the cell type. It has proved particularly useful in the identification of schwannian, muscular and myofibroblastic neoplasms; (4) Immunohistochemistry: this method has represented, without question, the most significant advance in the histologic interpretation of sarcomas; (5) Additional techniques which are beginning to be applied on a consistent basis and which promise to provide information of importance include: chromosomal studies, analysis of DNA ploidy, determination of cell cycle associated-nuclear proteins, and evaluation of oncogene expression. Preliminary data have shown some specific abnormalities, and some relationship between DNA ploidy and behavior.

CL 013 RHABDOMYOSARCOMA, EWING'S SARCOMA, AND NEUROECTODERMAL TUMORS OF CHILDREN: MODELS OF TUMOR MOLECULAR GENETICS, CYTOGENETICS, & CELL BIOLOGY, Timothy J. Triche, Department of Pathology and Laboratory Medicine, Childrens Hospital Los Angeles and University of Southern California, Los Angeles, CA 90027.

The diagnosis and treatment of malignancy is inextricably linked to the underlying biology of the tumor. Laboratory studies of childhood tumors, especially sarcomas, have been highly informative. My laboratory over the past decade has focused on two tumor systems in particular. The first major study focused on the origins of an enigmatic bone tumor, Ewing's sarcoma, originally of unknown origin and relationship. The second began as a study of why certain forms of childhood and young adult rhabdomyosarcoma appear to differ so greatly in their survival.

Ewing's sarcoma appears to be unrelated to the most common tumor, osteosarcoma. Until 1984, no specific histogenesis was known. A combination of cell biology, cytogenetic, molecular genetic, biochemical, and enzymatic studies of newly established tumor cell lines and fresh or frozen tumor tissue has definitively shown that at least 90% of Ewing's tumors studied to date (and probably 100%, given the vagaries of definitive diagnosis) are of potential or actual neuroectodermal phenotype. This knowledge led directly to the realization that many "metastatic" neuroblastomas, especially in bone, are not. Likewise, it became obvious that soft tissue sarcomas of "Ewing's" type are also (for the most part) neuroectodermal tumors. Surprisingly, knowledge of the expected results by the techniques noted above has further extended the "Ewing's" neuroectodermal phenotype to other tumors as well, including small cell osteosarcoma and a group of ill-defined spindle cell sarcomas of bone. This is not true of conventional osteosarcoma, however.

Rhabdomyosarcoma appears in two major forms, embryonal (ERMS) and alveolar (ARMS). ERMS survival exceeds 80%, while ARMS approaches 25%. We have established &/or characterized cell lines from both ERMS and ARMS, including particularly aggressive primitive forms of the latter ("solid" ARMS). Unlike the Ewing's sarcoma/PNET rcp(11:22) chromosomal translocation, we do not find a consistent chromosomal marker of A vs. E RMS, or even RMS for that matter (despite the occurrence of a t(2:13) in some lines). We do find a consistent difference in xenograft tumorigenicity, metastatic potential, and constitutive quantitative overexpression of c-*myc*, a proliferation associated oncogene. We are currently exploring underlying causative factors, including a potential role for RB1, the retinoblastoma tumor suppressor gene, in this tumor system.

In both of these studies, the results to date have fundamentally altered conventional pathologic and clinical thinking in connection with these tumors. Future refinements in diagnosis and treatment will depend on further laboratory studies, especially of fresh tumor tissue. These should be adopted in the diagnostic evaluation whenever feasible.

Molecular Cytogenetics

CL 100 MAPPING THE X;18 CHROMOSOME TRANSLOCATION IN HUMAN SYNOVIAL SARCOMA, Jennifer C. Knight, Jeremy Clark and Colin S. Cooper. Department of Pathology, Institute of Cancer Research, London SW3 6JB. Human synovial sarcoma tumours show a characteristic primary chromosome translocation, t(X;18)(p11.2;q11.2). We have been screening DNA from synovial sarcomas with probes to genes known to be located in the region Xp11.2 to Xp11.4 that spans the translocation. So far there is no evidence for rearrangements or altered expression of the ARAF1 proto-oncogene, TIMP, Elk1 A1S9 or SB1.8 in the four tumours examined by Northern and Southern blotting, including long range restriction enzyme analysis. While continuing to screen tumours with probes to known gene loci we are also using reverse genetics to map the chromosome breakpoint. From one tumour we derived two somatic cell hybrid lines containing derX only and have previously reported that the two markers that appear to be closest to the X;18 junction in this tumour are DXS14 and DXS146. Irradiation of a hamster cell line CL12D carrying a normal human X chromosome yielded a hybrid containing a DXS14/DXS146 fragment. From this hybrid line we have generated a Lambda phage library to saturate the DXS14/DXS146 region with new probes for fine detailed mapping. These probes may be used to identify and clone rearranged DNA sequences in the hybrid lines derived from synovial sarcomas.

CL 101 IDENTIFICATION AND CLONING OF SOMATIC DNA ALTERATIONS IN SOFT TISSUE SARCOMAS. Kelly Kopald, Toyoaki Uchida, Stephen Kallaos, James Economou, Frederick Eilber, and Harry Neuwirth, Department of Surgery, Divisions of Surgical Oncology and Urology, UCLA School of Medicine, Los Angeles, CA 90024. A number of mechanisms at the genomic level are known to be involved in the development of neoplasms including mutation, rearrangement, and amplification. We used DNA fingerprint analysis as a first step toward the identification of genomic abnormalities in soft tissue sarcomas. DNA was isolated from soft tissue sarcomas and peripheral blood leucocytes in 13 patients. Tumor cell types included: liposarcoma (4), leiomyosarcoma (3), malignant fibrous histiocytoma (2), mesenchymoma (1), spindle cell sarcoma (1), neurofibrosarcoma (1), and dermatofibrosarcoma (1). The DNA was digested to completion with Hae III and Hinf I restriction enzymes, Southern blotted, and probed with a radiolabelled DNA minisatellite probe recognizing highly repetitive DNA sequences. The autoradiographs were analyzed for four types of alterations in the soft tissue sarcoma DNA compared to the control DNA, including: band deletions, decreased band intensity, new bands, and increased band intensity. All of the soft tissue sarcomas exhibited one or more band changes when compared with the constitutional DNA. Using the Hae III restriction enzyme, 5 of 13 tumors revealed band deletions or decreasing intensity in one or more bands and 6 of 13 tumors had new bands or amplified bands. With the Hinf I digest, 5 of 13 tumors had deletions or decreasing band intensity and 5 of 13 tumors clearly showed new bands or increases in band intensity. Two tumors (one liposarcoma and one neurofibrosarcoma) exhibited one or more highly amplified bands when compared with the normal DNA. We have successfully cloned one of the amplified regions from the liposarcoma which will permit determination of how these amplified DNA sequences affect neoplastic development and tumor progression. We conclude that DNA fingerprint analysis is a useful method for identifying changes in the somatic DNA of soft tissue sarcomas. Identification of highly amplified regions in the sarcoma DNA suggests that clonal alterations were found in the majority of cancer cells.

CL 102 DETECTION OF LOSS OF HETEROZYGOSITY IN EMBRYONAL RHABDOMYOSARCOMA USING POLYMERASE CHAIN REACTION, Mark A. Lovell, Linda L. Gilmer, Department of Pathology, University of Virginia Health Sciences Center, Charlottesville, VA 22908.
In tumors loss of heterozygosity (LOH) is often associated with loss of a tumor suppressor gene. LOH at 11p15.5 has been reported as a consistent alteration in embryonal rhabdomyosarcoma (Scrable H. et al, PNAS 86: 7480-4, 1989). The H-ras proto-oncogene in this chromosomal subband is polymorphic due to a variable number tandem repeat (VNTR) at its 3' end. Using flanking primers on either side of this VNTR region in a polymerase chain reaction (PCR), specimens from three cases of embryonal rhabdomyosarcoma were studied in comparison to corresponding normal white blood cell DNA. In two of three cases LOH was directly evident on ethidium bromide stained gels. In all three cases findings corresponded to results obtained using classic RFLP analysis of unamplified genomic DNA by Southern blot at this locus. This result suggests that the PCR method is applicable for detection of LOH in clinical tumor specimens, which would permit rapid study of small biopsies or archival specimens. Further studies utilizing this method are in progress in our laboratory.

CL 103 STRATEGIES FOR GENERATING CHROMOSOME 22-SPECIFIC PROBES, Ketan Patel, Vikki Groves, Janet M. Shipley, Garret Hampton, Peter Riddle and Denise Sheer. Imperial Cancer Research Fund, P.O. Box 123, Lincoln's Inn Fields, London WC2A 3PX, U.K.

Both Ewing's sarcomas and Askin's tumours show a consistent (11;22)(q24;q12) chromosome translocation. In order to map the breakpoint and isolate the gene(s) responsible for the generation of these tumours, informative markers are required with which to approach the breakpoint. As yet the 22q12 region remains ill-defined as does most of the long arm of this chromosome. We have devised three strategies to generate probes on chromosome 22 and to employ these to map the breakpoint region.

The first method used PCR to amplify DNA from a chromosome 22 specific library using primers against the vector cloning site. The amplified products are screened from repetetive elements, and sequences of interest are analysed by *in situ* hybridisation and southern blotting on a hybrid panel. Using this technique single copy fragments of up to 8 kb have been generated.

We have made use of repetetive elements found dispersed in the human genome to generate human specific probes by using human specific primers to PCR DNA from somatic cell hybrids containing either chromosome 22 or a fragment of it as its sole human constituent.

The third and potentially most powerful technique involves microdissecting fragments of DNA from metaphase spreads and to use PCR to amplify DNA from the dissected regions. This method will be employed to dissect the breakpoint in translocations of interest.

Gene Expression, Alteration, and Amplification

CL 200 DETERMINATION OF CELL LINE IDENTIFICATION BY DNA FINGERPRINT ANALYSIS, Vivian H. Cohn, Jeannie Chen, Anna De Chiara, Laura Oslund, Leslie P. Weiner, Timothy J. Triche, and Peter J. Syapin, Department of Pathology, Childrens Hospital of Los Angeles, Los Angeles, CA 90027 and Department of Neurology and Cancer Research Center, University of Southern California School of Medicine, Los Angeles, CA 90033.

Cell lines can be a permanent resource and a valuable model for biological studies, and are especially useful when the availability of primary tissue is limited. However, reports of cross-contamination between cultures are becoming more prevalent. Such contamination, which may occur during their establishment or later handling, can result in cell lines unrelated to their source material. It is important, therefore, to verify the identity of lines. DNA fingerprinting offers a way of quickly assessing a unique identification. We have compared the DNA fingerprint patterns of several cell lines, including three rhabdomyosarcoma lines, CT10, TC212, and RD, and the medulloblastoma line, TE671. The patterns of CT10 and TC212 were identical, as were the patterns of RD and TE671. It is highly unlikely that the pattern similarities were due to random chance. RD and TE have been further examined for expression of the muscle marker, MyoD1, and both are found to express it. Additionally, when they were examined by immunofluorescence, myosin heavy chain was detected in both. Also, the original tumor tissue from which RD was established was shown to express a number of muscle specific gene products, including skeletal muscle myosin, desmin, and myoglobin. Thus, it appears likely that TE671 is, in fact, a subline of RD, and that RD and TE671, and CT10 and TC212, therefore, represent only two unique cell lineages. Work is currently underway to examine original tumor material to determine unambiguously the origin of these cell lines. These data clearly document the definitive role of DNA fingerprinting in determining lineage in established cell lines of any origin.

CL 201 THE ROLE OF DOMINANT AND RECESSIVE MUTATIONS IN SOFT TISSUE SARCOMA DEVELOPMENT. Colin S. Cooper, Sandra Moss, Barry.A. Gusterson, Cyril Fisher, Jennifer C. Knight, Brian R. Reeves, Christopher D.M. Fletcher, and Michael R. Stratton. Institute of Cancer Research, London, U.K. Previous studies have demonstrated that genes of the ras family (H,K and N) can be activated by point mutations at codons 12,13 and 61 in several classes of human cancer. In the present study we have used oligonucleotides corresponding to these regions to assess the possible role of ras gene mutations in the genesis of human rhabdomyosarcoma. The results show that 35% (5/14) of embryonal rhabdomyosarcomas contain activating mutations in N-ras or K-ras genes. The role of oncogene activation in other types of sarcoma was assessed by transfection of tumour DNA into NIH3T3 mouse fibroblasts. In these studies an activated K-ras gene was detected in a leiomyosarcoma while oncogenes unrelated to ras were detected by transfection of DNA from two liposarcomas.

Deletion or inactivation of cellular suppressor genes, such as RB-1 and p53, has also been implicated in the development of some classes of human cancer. To assess the role of alteration of these genes in soft tissue sarcoma development Southern blots of tumour DNA were hybridized to RB-1 and p53 gene probes. These experiments demonstrated the loss or gross rearrangement of the p53 gene in three sarcomas. Remarkably two of these three tumours had also lost both copies of the RB-1 suppressor gene indicating that one mechanism of sarcoma development may involve coincident inactivation of both p53 and RB-1 genes. This work was supported by grants from the Cancer Research Campaign and Medical Research Council.

CL 202 GLUTATHIONE PEROXIDASE (GPO) AT MDR-1 GENE EXPRESSION IN VITRO INDUCED DRUG RESISTANCE IN OSTEOSARCOMA. Byron P. Croker, Cheryl Zack and Ramy Saleh, Gainesville VAMC and Departments of Pediatrics and Pathology, University of Florida, Gainesville, FL. Most of the deaths from osteosarcoma are due to drug resistant tumors. Drug resistance was induced in the 791T osteosarcoma cell line by intermittent or continuous exposure to doxorubricin (Dox) in escalating doses to produce two sublines. The 50% inhibitory concentration of Dox (IC50) was increased 10-100 fold and abrogated by Verapamil and buthionine sulfoximine. Surface MDR-1 expression using the MRK 16 monoclonal antibody was increased 2.8 to 3.4 times and total cellular thiols were increased by 40%. The cell lines were cultured without drug and then given an oxidative challenge. GPO and mdr-1 mRNA were determined by RNA slot blot and Northern analysis after challenge and normalized to beta actin mRNA. GPO and mdr-1 were overexpressed and/or more rapidly inducible in the resistant cells. DNA was determined by DNA slot blot and Southern analysis. GPO was amplified 4-6 times and mdr-1 was amplified 6-10 times. We expect these studies will be important in understanding the mechanisms of drug resistance in osteosarcoma.

CL 203 THE ROLE OF RAS ONCOGENES IN LUNG CARCINOMAS IN HONG KONG, Maria Lung[1], Maria Wong[2], Wah Kit Lam[3], Susan Kwan[5], Henry Cheung[4], Wing Wei Yew[5], and Kin-hang Fu[2], Departments of Microbiology[1], Pathology[2], Medicine[3], and Surgery[4], University of Hong Kong, Hong Kong and Grantham Hospital[5], Hong Kong.

In Hong Kong lung cancer is the most common lethal malignant disease in both sexes. The mortality rate for lung cancer in Hong Kong was ranked medium in the world for men and the highest in the world for women: There is a preponderance of adenocarcinoma. Point mutations in codon 12 in the Kras oncogene have been reported in 5 of 10 bronchial adenocarcinomas in the Netherlands (Rodenhuis et al, 1987) and 2 of 27 lung carcinomas in the US. (Yanez et al, 1987).

We are presently investigating the incidence of point multational activation of Kras in lung carcinomas in Hong Kong by PCR and dot blot analysis and by Southern blotting techniques. The preponderance of adenocarcinomas and the high incidence of the tumor in nonsmokers in Hong Kong makes it of special interest.

CL 204 MALIGNANT TRANSFORMATION OF HUMAN FIBROBLASTS BY TRANSFECTION OF ONCOGENES, J. Justin McCormick, John E. Dillberger, Peter J. Hurlin, Daniel M. Wilson, Dennis G. Fry, and Veronica M. Maher. Carcinogenesis Laboratory, Michigan State University, East Lansing, MI 48826-1316.
Human fibroblasts in culture have not been successfully transformed to malignancy by carcinogens, even though data indicate that carcinogen exposure is the cause of most human tumors. To understand why human fibroblasts in culture are so refractory, we transfected them with oncogenes active in transformed rodent fibroblasts or in tumorigenic fibroblasts derived from human fibrosarcomas. We found that activated H- or N-ras genes, flanked by suitable enhancer and promoter sequences, caused normal human fibroblasts to express many transformed phenotypes, but the cells did not acquire an infinite lifespan and were not tumorigenic. When we transfected H- or N-ras oncogenes in the same constructions used above or the v-K-ras gene into an infinite lifespan, near-diploid, non-tumorigenic human cell strain developed in this laboratory and designated MSU-1.1, distinct foci were observed. Cells from these foci formed progressively growing, invasive sarcomas in athymic mice. The tumors were characterized as spindle cell, round cell, or myxoid sarcomas, pleomorphic sarcomas with giant cells, or mesenchymomas. The various transfected ras oncogenes gave rise to specific tumor types, but since each ras gene was carried in a different plasmid construct, the role of the oncogene and the surrounding sequences in determining the specific type of tumor are not yet clear.

CL 205 SPONTANEOUS TERMINAL DIFFERENTIATION OF A HUMAN RHABDOMYOBLAST LINE IN VITRO, D.N. Shapiro, P.J. Houghton, B.J. Hazelton, G.S. Germain, K.G. Murti and J.A. Houghton, Departments of Hematology/Oncology, Pharmacology and Virology, St. Jude Children's Research Hospital, Memphis, TN 38101

Skeletal muscle differentiation consists of an ordered withdrawal of committed cells from the cell cycle and their fusion to form multinucleated myotubes. To determine if differentiation of malignant myoblasts parallels that of normal skeletal muscle, a cell line (Rh28) was established from an alveolar rhabdomyosarcoma bearing the characteristic t(2;13) translocation. Rh28 displays a constant population doubling time of 45-55 hrs until passage 60 when the doubling time progressively increases until spontaneous division ceases by passage 70. Rh28 cultures in early passage are characterized by small round cells with sparse cytoplasm whereas late passage cultures consist of large, multinucleated cells with cytoplasmic fusion. Late passage cells also demonstrate organization of actin filaments into myofibrilar arrays. Morphologic differentiation is accompanied by the temporal expression of genes associated with myotube development including desmin, δ- and γ- subunits of the acetylcholine receptor, muscle-specific creatine kinase, myosin heavy chain, and skeletal muscle actin. To examine if multinucleated cells in late passage Rh28 cultures are quiescent and thus similar to myotubes formed by the fusion of normal myoblasts, thymidine labeling was performed. Multinucleated cells incorporated label uniformly, suggesting synchronous replication and cell cycle arrest in G_2 since cell number did not increase. These data demonstrate that Rh28 undergoes spontaneous terminal differentiation analogous to normal myoblasts and thus will be an important model for understanding the behavior of malignant myoblasts.

CL 206 MOLECULAR MECHANISM AND DETECTION OF MULTIDRUG RESISTANCE IN SARCOMAS. J.S. Wunder, S. Keating, A.A.Czitrom, I.L. Andrulis, Mount Sinai Hospital Research Institute,University of Toronto,Toronto,Canada, M5G1X5; C.Beck, K.E. Noonan, I.B. Roninson, Dept. of Genetics, University of Illinois at Chicago, Chicago, Illinois,60612

Resistance to combination chemotherapy remains the major cause of treatment failure in osteosarcomas and soft-tissue sarcomas, yet cannot be adequately assayed for in these tumors. Simultaneous cross-resistance to unrelated lipophilic cytotoxic agents including anthracyclines, epipodophyllotoxins and Vinca alkaloids commonly affects sarcomas and is termed multidrug resistance(MDR). MDR results from increased expression of the mdr gene family which includes two linked genes, mdr1 and mdr2. Only mdr1 expression has been associated with MDR, and its detection may be an important predictor of clinical response to lipophilic chemotherapy. Initial slot blot results suggested that the majority of sarcomas have low or no mdr1 expression. To increase the sensitivity of our assay, we used polymerase chain reaction(PCR) mediated amplification of cDNA sequences corresponding to different mRNAs to measure both mdr1 and mdr2 expression. mdr1 expression was detectable in the majority of osteosarcomas(24/31) and soft-tissue sarcomas(47/62) and was intrinsic in most of these as they had not received pre-operative chemotherapy. Ewing's sarcomas, tumors which are generally the most chemosensitive of the group, expressed no mdr1 mRNA, suggesting that slightly increased mdr1 levels detectable by PCR in other sarcomas may be clinically relevant. Expression of mdr2, which is not associated with MDR, was found in 90% of sarcomas. These results are being correlated with clinical data. Detection of mdr1 expression may be helpful in identifying and individualising treatment for MDR sarcomas.

Growth Factors

CL 300 MALIGNANT TRANSFORMATION OF HUMAN FIBROBLASTS CORRELATES WITH HIGH LEVELS OF ACTIVITY OF A MEMBRANE-BOUND UROKINASE, Jerzy Jankun, Veronica M. Maher, J. Justin McCormick, Carcinogenesis Laboratory, Michigan State University, East Lansing, MI 48824-1316.

By transfection of suitable oncogenes or carcinogen treatment, and sequential clonal selection, we have developed a cell lineage that begins with normal diploid human fibroblasts, has several members with progressively more transformed phenotypes and concludes with malignant fibroblasts. We have used the series of cells in this lineage to test the hypothesis that transformation of cells to the malignant state includes an increased expression of plasminogen activators (PAs). PAs were measured as urokinase PA (u-PA) or tissue PA (t-PA) activity that was bound to the cell surface as well as those secreted into the medium. The activity of the membrane-bound u-PA was 3-8 times higher in malignant cells than in non-malignant precursor cells. Receptor bound t-PA, as well as secreted u-PA and t-PA were generally higher in the malignant cells than in precursor cells, but there were some inconsistencies. Membrane-bound u-PA activity was also higher in the several human fibrosarcoma-derived cell lines that were examined than in the precursor cells suggesting that the in vitro model we are using for malignant transformation mimics the in vivo process of cell transformation. (Supported by DOE Grant 60524, NCI Grant CA21289, and NIEHS Contract ES 65152.)

CL 301 CLONAL VARIATION OF EXPRESSION OF THE GENES CODING FOR PLASMINOGEN ACTIVATORS (PA) AND THEIR INHIBITORS (PAI) IN HT1080 SARCOMA CELLS.
Walter E. Laug, William Rideout, Rita Mundy, Emil Bogenmann, and Kai Wang*. Childrens Hospital of Los Angeles, Los Angeles, CA, USA, and *Division of Biology, California Institute of Technology, Pasadena, CA, USA.

The human sarcoma cell line HT1080 was found to consist of clones secreting either high (secretor clones) or low levels (nonsecretor clones) of PA. The uncloned parental cell line was shown to express both urokinase (uPA) and tissue type PA (tPA) genes and the 3 PAI genes coding for PAI-1, PAI-2 and protease-nexin I. Northern blot analysis of the different clones revealed considerable variation of expression of the different PA and PAI genes respectively, with the secretor clones expressing mainly the PA genes whereas the nonsecretor clones demonstrated high expression of the PAI genes. These findings were verified with *in situ* hybridization using specific uPA and tPA riboprobes. Gene amplification or loss was excluded by Southern blotting. Protein determinations with monospecific antisera in serum-free medium conditioned by the various clones coincided with the RNA findings. This suggests differences either on the level of transcription or in RNA processing and/or stability. Growth of the various HT1080 clones on extracellular matrices demonstrated an increased protein degradation by the secretor clones compared to the nonsecretor clones.

These findings demonstrate considerable heterogeneity of PA and PAI gene expression within the HT1080 cell line. We speculate that the cells expressing rather high levels of the PA genes are those that invade and degrade surrounding tissues, whereas those expressing mainly PAI are responsible for the structural composition of tumors. This hypothesis is currently investigated with *in situ* hybridization on freshly removed human tumors using specific riboprobes.

CL 302 BINDNG OF HEPARAN SULFATE (HS) BY RHABDOMYOSARCOMA CELLS IS DEPENDENT ON THE METASTATIC PHENOTYPE.
Elemer Moczar, Madeleine Moczar, Daniel Roulais* , Marie-France Poupon**, and Yves Courtois*. Lab. de Biochimie du Tissu Conjonctif, F-94010 Créteil; *Inst. Res. Gérontologiques, F-75016 Paris; **IRSC, CNRS F-94804 Villejuif. Rat rhabdomyosarcoma cells with high (RMS0) and low (RMS8) metastatic capacity exhibit differences in binding and internalization of heparan sulfate (HS), (Redini et al. Bichem. Biophys . Acta, 991, 359. 1989). Here the proteins able to bind specifically to iduronate containing glycosaminoglycans (GAG)in the RMS cells were studied. Proteins were isolated from plasma membranes and from preparations enriched in inner membranes and nuclei by affinity chromatography on Heparin-Ultrogel. Heparin eluted from the affinity column a mixture of 4 proteins in a molecular weight range of 15-22 KD, able to stimulate the growth of bovine epithelial lens cells. This effect was due to a minor 22 KD component reacting on Western blots with antiserum to basic fibroblast growth factor (bFGF). The affinity of sulfated GAGs to the main 19 KD component are decreasing in the following order: heparin> HS >dermatan sulfate >>chondroitin sulfate. Accordingly, the polysacharide structure, as the block regions containing sulfated iduronate and N-sulfate groups were determinant factors for the interactions. In agreement with the previously observed decreased receptor mediated binding of HS, the highly metastatic line expressed two times less HS binding proteins on cell number basis, than the low metastatic counterpart. A negative correlation seems to exist in this model between the expression of the metastatic phenotype and the 22 KD form of the bFGF able bind to HS.

CL 303 HYALURONATE BINDING PROTEINS IN HIGHLY METATSTATIC RHABDOMYOSARCOMA CELLS
Madeleine Moczar and *Marie-France Poupon, Laboratoire de Biochimie du Tissu Conjonctif,CNRS UA 1174, 94010 Créteil, *Laboratoire des Métastases CNRS IRSC, 94800 Villejuif, France. Hyaluronate (HA) a glycosaminoglycan has been implicated in the regulation of cell adhesion, movement and differentiation. In sarcomas produced by virus (wt FVS) transformed Rat 2 cells, the accumulation of HA rich extracellular matrix was dependent on oncogene expression. The adhesion of trypsinised highly metastatic rat rhabdomyosarcoma (RMS0) cells, expressing myc and ras oncogenes could be enhanced by exogenous HA. The release of pericellular HA by trypsin suggested that the retention of HA by the cells was mediated by proteins.The proteins and HA were biosynthetically labelled in confluent cultures of RMS0 cells. The cells were fractionated and the HA binding proteins (HABP) isolated by affinity chromatography on HA-Sepharose. On HPLC the main HABP was of~80 kD mol. size in the cytoplasma and in the membrane fractions. From the pericellular domain and the medium, the polypeptides in mol. weight range 40-14kD were coeluted with HA on ion exchange chromatography. The proteins eluted from the affinity column and the polypeptides cochromatographed with HA on DEAETrisacryl exhibited a specific binding to HA as it was shown by the competition assays with non labeled glycosaminglycans. The low molecular weight components indicated the proteolytic processing of the secreted HABP. HABPs may act as binding sites for the extracellular HA and thus can ensure the association of the HA rich matrix with rhabdomyosarcoma cells by specific protein-polysaccharide interactions.

CL 304 PDGF A AND B CHAINS AND PDGF α AND β RECEPTORS ARE DIFFERENTLY EXPRESSED IN HUMAN SARCOMA CELLS AND IN FIBROBLASTS; Monica Nistér, Ylva Paulsson, Tomas Söderman, Margareta Genberg, Joachim Mark and Bengt Westermark, Department of Pathology, University Hospital, S-751 85 Uppsala and the Cytogenetic Laboratory, Department of Pathology, Central Hospital, S-541 01 Skövde, Sweden. Growth factors and their receptors are expressed in malignant cells, often without signs of amplification or rearrangement of the corresponding genes. The PDGF A and B chain (c-sis) genes are known to be expressed in sarcoma cell lines and introduction of the sis oncogene has, in different experimental systems, induced fibrosarcomas. Two cell lines have been established from two successive recurrences of a subcutaneous malignant fibrous histiocytoma. The first cell line, U-2149, had a fibroblast-like morphology and a modal distribution in the hypotriploid region, while the second, U-2197, contained mostly histiocyte-like cells in the penta-hexaploid region. By banding analysis and identification of identical chromosomal marker types it was proven that the two lines were derived from the same single cell or single clone. The karyotype analysis explained the occurrence of the pleomorphic histiocyte-like cells in U-2197 as a consequence of chromosomal progression. While U-2149 expressed PDGF and PDGF receptor genes at approximately the same level as human fibroblasts, U-2197 contained high levels of PDGF A chain and PDGF-α receptor mRNA. The U-2149 cells contained increased levels of EGF receptor mRNA.The growth properties of the two cell lines in serum and in serum-free media will be described. Together with human fibroblasts they were used to investigate the regulation of PDGF and PDGF receptor genes by extracellularly added isoforms of PDGF in order to elucidate the nature of the constitutive expression described in malignant cells.