The Philadelphia Academy of Surgery

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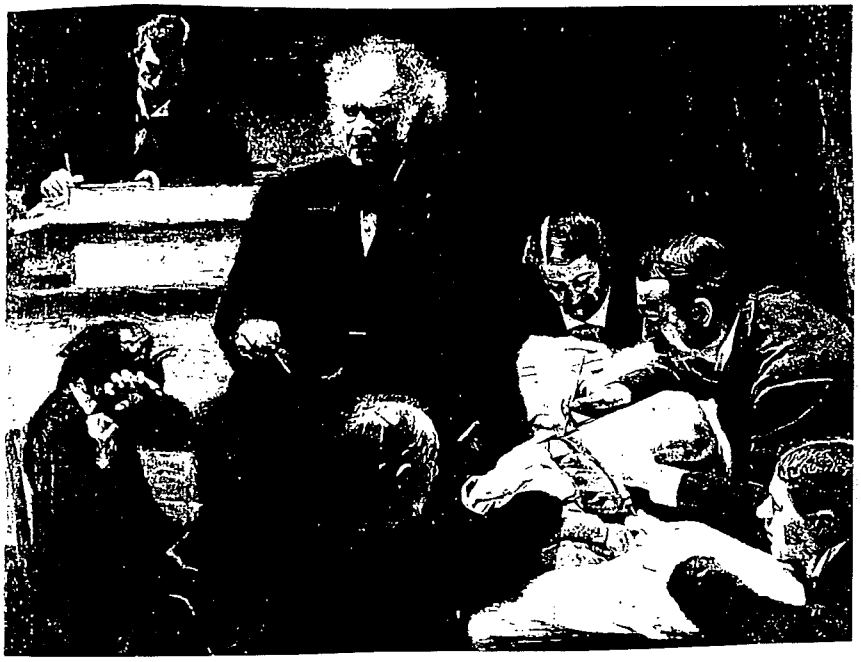
Resident Paper Presentation



Presentors:

Dr. Gary A. Tuma – "The Relationship between Human Preadipocytes and Mesenchymal Stem Cells"

Dr. David J. Maron – "The Role of NK Cells in the Treatment of Hepatic Micrometastases of Colorectal Cancer Using Adenoviral-Mediated Interferon-Beta Gene Therapy"

Dr. Joseph A. Blansfield - "Recent Experience with Preoperative Fine Needle Aspiration Biopsy of Thyroid Nodules in a Community Program"

In Memoriam

Dr. Caswell Taylor – presented by Dr. Robert Harwick

Dr. Bernard Siegel – Presented by Dr. Andrew Roberts

Title: The Relationship between Human Preadipocytes and Mesenchymal Stem Cells

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Recently, there have been many reports found in the literature describing the uses and characteristics of mesenchymal stem cells. Mesenchymal stem cells are undifferentiated fibroblast like pluripotential cells that can form bone, cartilage, muscle and adipose tissue when exposed to various differentiating media in vitro.[1] Currently, they are isolated from adult human bone marrow or fetal tissue. Human preadipocytes are fibroblast-like undifferentiated cells found within adipose tissue. These cells can be expanded in vitro without differentiation and under proper culture conditions accumulate lipid and develop an adipose-like morphology.[2] We hypothesize that preadipocytes have similar multilineage differentiation potential as human mesenchymal stem cells when exposed to appropriate conditions. In addition, adipose tissue may be a source for the harvest of mesenchymal stem cells.

Method: Adipose tissue harvested from a TRAM procedure was processed to isolate the preadipocyte component. The isolation of preadipocytes was performed by the protocol described by Rodbell.[3]

The tissue was subjected to a collagenase digestion buffer, filtered through a 210μm-mesh sieve, and the stromal vascular fraction (pellet) was treated with an erythrocyte-lysing buffer. Once isolated the preadipocytes were plated at a concentration of $1x10^4$. These cells were expanded in culture for 4 population doublings until $9x10^6$ cells were available for experimentation.

A portion of the cells were plated at a density of $5x10^3$ per cm^2 and exposed to culture conditions known to support osteogenic differentiation of human mesenchymal stem cells isolated from bone marrow. The osteogenic differentiating media consisted of DMEM, 10% fetal calf serum, 0.1μm dexamethazone, 50μm L-ascorbic acid 2-phosphate, and 10mM beta-glycerophosphate.

The control group was exposed to culture conditions known to promote lipid accumulation and adipose differentiation. This media was made up of DMEM/Ham's F12, 10% fetal calf serum and antibiotics.

The culture media was changed every 48-72 hours. After 25 days in culture the cells were stained for adipose differentiation by Oil-Red-O and deposition of calcium phosphate by the method of Von Kossa. In addition the cells were evaluated for alkaline phosphatase activity using photo spectrometric analysis as well as direct staining in vitro of the cells. The control for the alkaline phosphatase activity was a human osteoblastic cell line.

Results: After 14 days in culture the cells exposed to osteogenic media showed microscopic signs of bony deposition and by 25 days bone nodules were grossly visible in the culture dish.

The cells subjected to the adipogenic differentiating media were stained with Oil-Red-O which stains lipid red. There were lipid droplets accumulating intracellularly and coalescing to form larger lipid vacuoles. These were absent in the cells exposed to the osteogenic differentiating media.

Staining by the method of Von Kossa revealed numerous areas of calcium phosphate deposition in the wells exposed to the osteogenic differentiating media. These areas correlated with the bone nodules seen grossly. These darkly stained regions, seen in all views within the well, represent extracellular bony matrix and calcium phosphate deposition (Figure 1).

In the control cultures, no bone formation was detected but numerous cells showed lipid accumulation consistent with mature adipocytes (Figure 2).

The cells exposed to the osteogenic differentiating media had greater expression of alkaline phosphatase activity when compared to the cells exposed to the adipogenic differentiating media but less than the control osteoblasts. This difference was statistically significant. When the two cell populations where stained for alkaline phosphatase activity it was grossly evident that the cells exposed to the osteogenic media expressed alkaline phosphatase and the cells exposed to the adipogenic media did not (Figure 3).

Conclusion: We have demonstrated that human preadipocytes can undergo osteogenic differentiation and deposit an extracellular bony matrix consistent with calcium phosphate deposition under proper culture conditions. They also express alkaline phosphatase activity after thirty days exposure to an osteogenic differentiating media, which is a marker for osteogenic differentiation.

These findings raise the possibility that human preadipocytes are in fact mesenchymal stem cells. Since mesenchymal stem cells offer wide therapeutic potential in areas of tissue engineering and cellular therapy, preadipocytes from fat normally discarded after surgical procedures could also be used for these purposes.

The Role of NK Cells in the Treatment of Hepatic Micrometastases of Colorectal Cancer Using Adenoviral-Mediated Interferon-Beta Gene Therapy.

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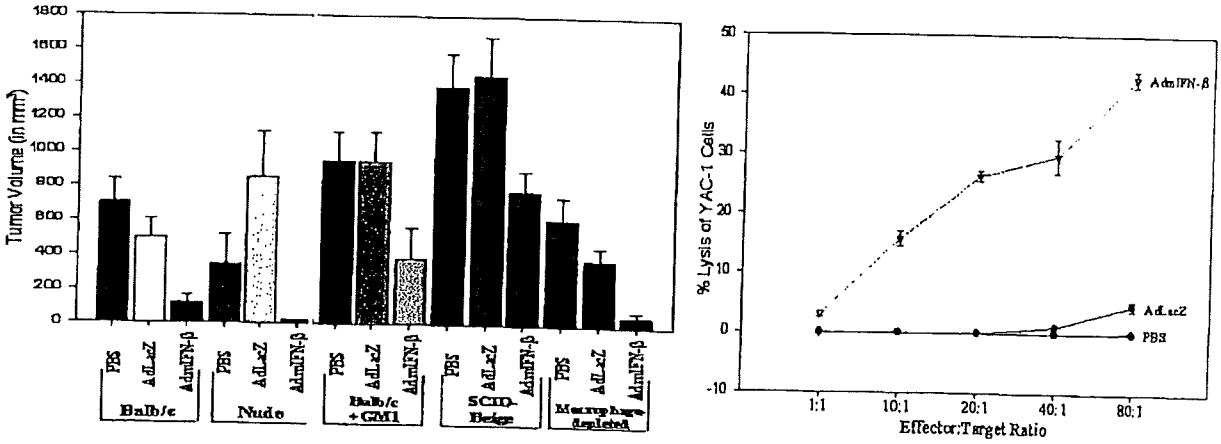
Introduction: We have been working to develop a treatment of hepatic colorectal metastases using systemically administered adenoviral vectors. We have previously demonstrated the effectiveness of adenoviral-mediated human interferon-β gene therapy in a colorectal liver metastases xenograft model. We sought to evaluate this in a syngeneic model to assess the role of host immune defenses. Experiments involving the murine colorectal cancer cell line CT-26 in an immunocompetent mouse and an adenovirus expressing murine IFN-β failed to show evidence of apoptosis as in the xenograft model, but showed similar evidence of tumoricidal activity. We hypothesized that a potential mechanism included the activation of the immune system by murine IFN-β.

Methods: Immunocompetent Balb/c mice, Nude mice (*nu/nu*), SCID-Beige mice, and Balb/c mice treated with anti-asialo GM1 serum (effective at depleting natural killer cells in mice) or liposome-encapsulated dichloromethylenediphosphonic acid (effective at depleting macrophages in mice) underwent a laparotomy during which 10^4 CT-26 cells were injected into the hepatic parenchyma. Five days later, the mice in each group were randomized to receive 5 x 10^{10} particles of AdCMVmIFN-β, 5 x 10^{10} particles of AdCMVLacZ (control vector), or 100 μl of PBS via tail vein injection. Fourteen days later mice were sacrificed and the livers were harvested for tumor measurement. In a separate experiment, Balb/c mice were sacrificed 3 days following administration of vector. Natural killer cells isolated from the liver and spleen using magnetic cell sorting were cultured for 48 hours in media supplemented with serum from the sacrificed animals. NK activity was then assessed via cytolysis of ^{51}Chromium-labeled YAC-1 murine hybridoma cells (a specific target of NK cells).

Results: Treatment with AdmIFN-β resulted in an 88% reduction in tumor volume ($p < 0.05$) in Balb/c mice, a 93% reduction in tumor volume ($p < 0.1$) in Nude mice, and a 91% reduction in tumor volume ($p < 0.05$) in macrophage-depleted Balb/c mice as compared with PBS and vector controls. AdmIFN-β treatment resulted in only a 60% reduction in tumor volume ($p < 0.05$) in Balb/c mice which were administered the anti-asialo GM1 serum and a 44% reduction in tumor volume ($p < 0.05$) in NK-deficient SCID-Beige mice as compared with PBS and vector controls. In addition, NK cells harvested from mice treated with AdmIFN-β showed a marked induction of cytolytic activity as compared with control animals.



Conclusion: Similar reductions in tumor volumes in the immunocompetent mice and Nude mice suggest that T-cell mediated immunity is not a dominant effector of inhibition of tumor growth in this model. Depletion of macrophages in Balb/c mice also did not affect the efficacy of AdmIFN-β treatment. Decreased effect of treatment in the NK-depleted (GM1) and NK-deficient (SCID-Beige) mice as well as induction of NK cell cytolytic activity following AdmIFN-β, however, suggests that natural killer cells may have a significant role in tumor killing following murine interferon-β gene therapy.

RECENT EXPERIENCE WITH PREOPERATIVE FINE NEEDLE ASPIRATION BIOPSY OF THYROID NODULES IN A COMMUNITY PROGRAM

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Background: Fine needle aspiration biopsy (FNA) has been used increasingly in the assessment and management of patients with thyroid nodules. Its acceptance has been slower in many community hospitals than in tertiary endocrine referral centers because reliable FNA requires expert cytopathologic assistance. We correlated preoperative FNA findings with postoperative histopathology to define the diagnostic accuracy and clinical utility of FNA in a community hospital setting.

Methods: FNA cytopathologic and final pathologic reports of all patients who underwent thyroidectomy at a community teaching hospital between March 1995 and March 2000 were analyzed to correlate post-thyroidectomy histopathologic and preoperative FNA cytopathologic findings.

Results: 281 partial or complete thyroidectomies were performed. 182 patients had a preoperative FNA and thyroid cancer was confirmed following thyroidectomy in 69 (38%) of them. FNA diagnostic of papillary carcinoma was reported in 28 patients with a predictive accuracy of 93%. FNA reports "suspicious" for papillary carcinoma" in 14 patients correlated with malignancy in 57% of patients. "Indeterminate follicular neoplasm" reported on FNA in 60 patients correlated with malignancy in 30% of patients, of whom 89% had papillary carcinoma (mainly follicular variant papillary carcinoma) and only 11% had follicular carcinoma. "Indeterminate Hürthle cell neoplasm" reported in 20 patients correlated with malignancy in 35% of patients. "Atypical cell clusters" reported in 5 patients and "Findings consistent with benign adenoma" in 4 patients in neither case correlated with any malignancy. "Benign" FNA findings in 40 patients who underwent thyroidectomy for other clinical features of their nodules correlated with malignancy in 20% of patients (5 of 8 patients had microcarcinoma < 1 cm). 18% of 11 patients who underwent thyroidectomy for "insufficient numbers of cells" after repeated FNA attempts had a carcinoma (2 of 2 patients had microcarcinoma < 1 cm). Nodule size with "indeterminate follicular neoplasm" reported on FNA analysis did not correlate with probability of malignancy and intraoperative frozen section analysis of such lesions was frequently inconclusive.

Conclusions: Accuracy of FNA analysis of thyroid nodules in a community hospital setting is comparable to results reported by major endocrine referral centers. Indeterminate findings of follicular neoplasm were the commonest FNA indication for thyroidectomy and correlated with the presence of differentiated thyroid cancers in 30% of patients.

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