and atypical MR and to correlate the phenotype and genotype.
METHODS: Exons 3 and 4 of the *MECP2* gene were analyzed by using denaturing gradient gel electrophoresis, sequencing, and gap polymerase chain reaction for (1) 124 children with FXS-like symptoms (102 boys, 22 girls) and 41 children with AS-like symptoms (14 boys, 27 girls) who tested negative for gene variation at the FXS and AS loci, respectively, (2) 23 girls with classical RS and 25 girls with atypical RS, and (3) 11 boys who were referred with possible RS. Statistical analysis (*t* and nonparametrical tests) included correlation of RS clinical severity score (Kerr, 2001) with *MECP2* mutations and frequency of *MECP2* mutations in the various patient categories.
RESULTS: Mutations were detected in 78.3% of classical and 20% of atypical RS cases, respectively. One boy carried the p.R106W mutation, and another boy showed a large rearrangement that required further characterization. Among AS- and FXS-like cases, 7.3% and 2.4% had *MECP2* mutations, respectively, including an X-linked MR case.
CONCLUSIONS: *MECP2* gene analysis provides an appropriate diagnostic tool for RS and contributes additional information for research into MR.

Hematology and Oncology

ASSESSMENT OF BONE MINERAL DENSITY AND MARKERS OF BONE TURNOVER IN CHILDREN UNDERGOING LONG-TERM ORAL ANTICOAGULANT THERAPY

Submitted by Maria Avgeri
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INTRODUCTION: Oral anticoagulants antagonize vitamin K action and potentially impair the carboxylation of osteocalcin, a protein that is essential for normal bone matrix formation.
OBJECTIVE: Our aim was to evaluate bone mineral density (BMD) and bone-turnover markers in 23 children who were undergoing long-term oral anticoagulant therapy (median age: 4 years) and 25 age- and gender-matched controls.
METHODS: BMD (characterized as a *z* score) of the lumbar spine was assessed by using dual energy radiograph absorptiometry. Osteoblast (bone alkaline phosphatase, osteocalcin, and amino-terminal procollagen 1 extension peptide) and osteoclast (urinary calcium and deoxypyridinoline and serum cross-linked C telopeptide) activity markers were measured. Vitamin D (25-hydroxy vitamin D, parathyroid hormone, whole and ionized calcium, phosphorus, and magnesium) and vitamin K (factors II, VII, IX, and X, protein C, protein S, and undercarboxylated osteocalcin [Glu-Oc]) statuses were determined.
RESULTS: Patients presented with higher levels of Glu-Oc, parathyroid hormone, and bone-resorption markers and lower levels of bone-formation markers and 25-hydroxy vitamin D; 52% of them showed signs of osteopenia ($-1.0 >$ BMD *z* score > -2.5). Statistical analysis demonstrated that anticoagulant therapy was an independent predictor of alterations in Glu-OC, osteocalcin, bone alkaline phosphatase, amino-terminal procollagen 1 extension peptide, and serum cross-linked C telopeptide levels.
CONCLUSIONS: Long-term use of coumarin derivatives may cause osteopenia in children with the risk of developing osteoporosis later in life.

IN VITRO ASSESSMENT OF MESENCHYMAL STROMAL CELL CHARACTERISTICS: IMPLICATIONS FOR THEIR CLINICAL USE

Submitted by Helen Dimitriou
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INTRODUCTION: Bone marrow (BM) stroma represents a source of progenitor stromal cells, termed mesenchymal stromal cells (MSCs), which are multipotent and can differentiate into cartilage, bone, and adipose tissue. Several questions have arisen regarding their long-term expansion and their safety before use.
OBJECTIVE: Our goal was to assess the long-term expansion and safety of MSCs in clinical practice.
METHODS: MSCs from BM of children with benign hematologic disorders and solid tumors without BM involvement were isolated and cultured for 10 consecutive passages (P). Immunophenotypic and functional characteristics, apoptosis, and the expression of cell cycle regulatory genes (*p53*, *p16*, and *Rb*) and signal transduction genes (*H-Ras*) involved in oncogenesis were assessed.
RESULTS: MSCs expressed mesenchymal-related surface antigens, >85% from P1. They had the ability to differentiate into osteocytes, adipocytes, and chondrocytes (reverse-transcription polymerase chain reaction). Colony forming units (fibroblast) ranged from 40.71 ± 4.3 at P1 to 15.5 ± 6.7 at P10. Their doubling time was 2.01 ± 0.14 days at P1 and 3.5 ± 1.19 days at P9. A low

percentage of apoptotic cells was detected (7-amino-actinomycin D [7AAD]) at P2 until P10. MSCs were resistant to apoptosis under serum-deprivation conditions. The expression of the cell cycle genes studied was not statistically different compared with controls, and cells did not grow on soft agar.

CONCLUSIONS: MSCs isolated from BM of children retain their characteristics for a serial number of passages and survive under serum-deprivation conditions, a necessary process in a transplantation setting. The cells do not have oncogenic properties, as shown by normal expression levels of oncogenes and tumor suppressor genes, and no growth on soft agar. These findings enhance the use of MSCs in clinical applications.

NDRG1 EXPRESSION IN CHILDHOOD LEUKEMIA AND ITS CORRELATION TO PROGNOSIS AND THERAPEUTIC RESPONSE

Submitted by Ju Gao

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INTRODUCTION: N-myc downstream regulated gene 1 (*NDRG1*) gene expression has been found to be downregulated in a variety of solid tumors and is now regarded as a suppressor gene. However, little is known about its possible role in hematologic cancers.

OBJECTIVE: Our goal was to study expression of the *NDRG1* gene in childhood leukemia and explore a possible correlation between expression and prognostic factors.

METHODS: Bone marrow or peripheral blood mononuclear cells from 65 children with leukemia and peripheral blood mononuclear cells from 12 healthy control children were isolated: *NDRG1* messenger RNA expression was determined by fluorescence real-time polymerase chain reaction.

RESULTS: *NDRG1* messenger RNA expression in acute leukemia groups collectively (acute lymphocytic leukemia [ALL] [41 cases] and acute monocytic leukemia [24 cases]) was significantly lower than that of normal controls (normalized ratios of *NDRG1* to glyceraldehyde-3-phosphate dehydrogenase copy numbers were 0.27 and 0.25 vs 0.30 and 0.86 in controls, respectively; $P < .01$), although there was no statistically significant difference between the ALL and acute monocytic leukemia groups. *NDRG1* expression was significantly lower in prednisone nonresponder ALL (13 cases) than in prednisone good-responder ALL (15 cases) (normalized ratios: 0.13 and 0.38, respectively). Similarly, *NDRG1* expression was significantly downregulated in high-risk ALL (17 cases) than that in lower-risk ALL (24 cases) (normalized ratios: 0.15 and 0.30, respectively).

CONCLUSIONS: *NDRG1* expression was remarkably downregulated in childhood leukemia, as in other human solid tumors. In addition, its expression in childhood ALL was closely associated with such prognostic factors as prednisone response and risk stratification. Our research suggests that *NDRG1* expression is negatively correlated to ALL prognosis and therapeutic response.

IMMUNE STATUS AND IMMUNE RECOVERY IN CHILDREN WITH LYMPHOMA AT THE END OF THERAPY (CHEMOTHERAPY AND/OR RADIOTHERAPY) AND IN FOLLOW-UP EVALUATIONS

Submitted by Helen Kosmidis

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OBJECTIVE: We aimed to evaluate the immune status and immune recovery after completion of chemotherapy and/or radiotherapy in children with lymphoma.

METHODS: We prospectively evaluated humoral and cellular immunity in 22 children with lymphoma (11 with Hodgkin's disease [HD] and 11 with non-Hodgkin's lymphoma [NHL]) at the completion of therapy and every 6 months thereafter.

RESULTS: Immunoglobulin (Ig) levels were normal before the onset of therapy in all but 1 child. At the end of therapy, Ig levels decreased: IgM in 18, IgG in 12, and IgA in 7 children. In addition, 17 of 22 had decreased CD19 levels. In HD after radiotherapy, IgG and CD19 levels increased significantly ($P = .013$ and .004, respectively). IgM levels remained abnormally low in 16 of 22 children up to 18 months after therapy completion. At the end of therapy, helper T lymphocyte (CD4) levels were low in 20 of 22 children, and suppressor (CD8) levels were elevated in 13 of 22 children. (For those with HD before radiotherapy, the CD8 level was high in 10 of 11 children, and the CD4 level was low in 6 of 11 children.) The suppressor CD8 level remained elevated in 12 of 20 children, and helper CD4 level remained abnormally low in 18 of 20 children for a period of 6 to 18 months after therapy. Some immunized children became nonimmune to polio (15 of 22), mumps (6 of 22), rubella (5 of 22), and measles (1 of 22).

CONCLUSIONS: In children with lymphoma, IgM levels remained low for long periods. Helper T lymphocyte levels were low and suppressor levels were

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