Gene Expression Analysis of Osteoblast-related Factors in Human Bone Marrow Cells from Patients with Total Hip Replacement

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INTRODUCTION

Prosthesis longevity after total hip replacement (THR) requires an efficient periprosthetic ossification. This includes the recruitment of bone marrow stromal cells (BMSC) to the site. The expression of the genes for alkaline phosphatase (ALP), Runx2, MSX2, bone morphogenetic protein 2 (BMP2), and two of its receptors (BMPR-1a and BMPR-1b) indicates the bone forming potential of a population of BMSC. We propose that a comprehensive analysis of the bone marrow osteogenic potential is helpful in predicting the strength of prosthesis fixation. The purpose of this pilot study was to investigate the expression of osteoblast-related genes in BMSC derived from human bone marrow aspirate and the possible linkage of these gene expression levels with patient age, sex and body mass index (BMI) in a group of THR patients.

MATERIALS AND METHODS

The study was approved by the Institutional Review Board at Providence Hospital. BMSC were prepared from bone marrow aspirate obtained from the proximal femur metaphysis during THR surgery. Twelve patients were included in this study (8 men and 4 women, ages 50-84, BMI 21.5 - 50). The isolated BMSC were culture-expanded in basal αMEM/10% fetal bovine serum medium. Passage 1, non-confluent, undifferentiated cells were harvested for flow cytometry or for RNA isolation. Real time RT-PCR with Taqman or SYBR green was used to measure the mRNA level of genes (SOST, ALP, Runx2, MSX2, BMP2, BMPR-1a, and BMPR-1b).

RESULTS

The expression of osteoblast-relevant genes varied greatly in these 12 patients (Table 1). Statistical analysis using SPSS showed no significant relationship between osteogenic markers and either age, sex or BMI (data not shown).

Table 1	mRNA expression normalized with GAPDH (2^-delta CT*1000)						
Sample #	SOST	ALP	Runx2	MSX2	BMP2	BMPR-1a	BMPR-1b
1	0.00863	17.18070	1.76441	3.46592	0.47728	1.52743	0.17611
2	0.00917	51.23903	3.99833	7.79673		2.87630	0.05225
3	0.00096	5.29146	0.52063	1.91247	0.53067	1.12338	0.13665
4	0.00183	2.35743	0.53806	2.11196	0.50636	1.37939	0.43358
5	0.00241	3.25933	1.41997	4.10263	0.78139	2.44128	0.21639
6	0.00087	5.64386	0.51756	1.42630	0.01574	0.68555	0.27183
7	0.00074	3.14547	0.54518	1.77314	0.04124	0.89855	0.14950
8		3.46355	0.99121	5.95253	0.83870	1.20698	0.33754
9	0.00082	0.36927	1.44043	1.59329	0.17993	0.44839	0.15108
10	0.00137	1.07500	2.20714	1.98043	0.24308	0.63925	0.08496
11	0.00474	5.83982	2.01673	1.64856	0.17977	0.67222	0.04953
12	0.00086	24.63173	4.31428	2.89751	0.13911	0.76971	0.40227

Linear regression analysis of the gene expression of BMP2 and BMPR-1a demonstrated a significant relationship (P<0.01) (Fig. 1), whereas there was no significant relationship between BMP2 and BMPR-1b (data not shown).

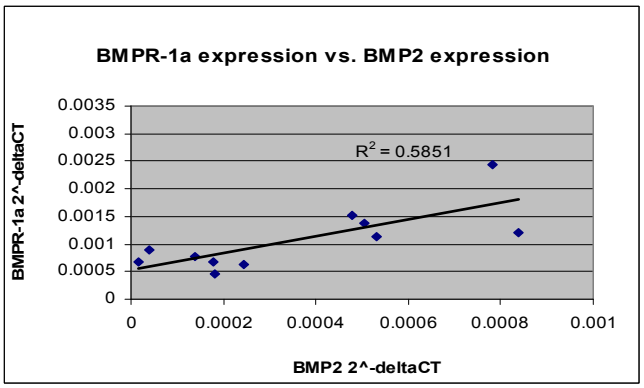

Figure 1 Correlation between BMP2 and BMPR-1a expression in Passage 1, non-confluent, undifferentiated BMSC from THR patients.

There was also a significant positive association between SOST mRNA expression and ALP, MSX2 and BMPR-1a mRNA expression (Table 2). Detection of the cell surface marker Stro1 by flow cytometry confirmed the mesenchymal cell characteristics of the BMSC (Table 2).

Table 2	Gene expressed						
	SOST	ALP	Runx2	MSX2	BMP2	BMPR-1a	BMPR-1b
SOST mRNA R^2		0.435	0.095	0.49	-0.02	0.333	0.068
P value		0.016	0.186	0.01	0.391	0.037	0.222
%Stro1+ cells R^2	0.475	0.917	0.387	0.441	-0.071	0.342	-0.02
P value	0.011	0.001	0.018	0.011	0.575	0.027	0.397

CONCLUSION

A patient's osteogenic potential at the time of joint replacement may determine the success of the implant fixation. Although a patient's sex, age or BMI did not correlate with osteogenic potential, all may impact the success of the implant. Moreover, given the probability that some implants will fail, the wide variation in mRNA expression of osteogenic-relevant genes also provides an opportunity to correlate gene expression with the likelihood of implant failure. Further study with these BMSC cultures should validate the use of these osteogenic markers in predicting osteoblastogenesis and potentially prosthesis success.

REFERENCES:

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