Tumor suppressor gene alterations of spontaneously malignant transformed cells from human embryonic muscle in vitro

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Abstract. Recent research has shown that mesenchymal stem cells (MSCs) which were cultured for long time could transform malignantly, the transformation mechanism is not clear yet, it might be associated with the activation of oncogenes and inactivation of tumor suppressor genes. In our initial investigation, we found that the cells arising from human embryonic muscle could spontaneously transform into malignancy in vitro and we obtained 6 immortalized cell lines. In this study, polymerase chain reaction (PCR) was used to assay several tumor suppressor genes of these cell lines, and homozygous deletions within chromosomal band 9p21 including MTAP (methylthioadenosine phosphorylase), p16 and p15 were detected. PCR products of p53 exons 7 and 8 of these novel tumor cell lines were assayed by sequencing, and the results showed high prevalence of mutations in these regions, the mutation rate reached as high as 8% in exon 7 and 14% in exon 8, and all of them were point mutations, the intron 7 changed more significantly, including piece deletion, insertion, frameshift and point mutation, it showed almost no similarity to that of the wt p53 sequence, that was totally different from other p53 mutation data published. All the mutation sequences were identical in 6 cell lines, this suggest that there may be a common mutation mechanism and strong selective advantage in these novel tumor cell lines over long-term culture. In conclusion, our research shows that the inactivation of tumor suppressor genes may play an important role in the process of malignant transformation of embryonic muscle cells in vitro.

Introduction

The development of tumors is generally accepted to be a multistep process in which alterations in oncogenes and tumor suppressor genes play an important role (1,2). The process of carcinogenesis involves the gain of oncogene activity and the loss of tumor suppressor gene function, such as Rb, p53 and cyclin-dependent kinase inhibitor (CDK) family (3-5). The aberrant gene could interfere in many cell functions, such as cell proliferation, differentiation and apoptosis. The cumulative mutations constitute the base of cell malignant transformation in gene level. The acquired capabilities of tumor cells include their ability to proliferate continuously ignoring apoptosis or growth-inhibitory signals, generating their own mitogenic signals.

At present, the reports on the malignant transformation of human cell in vitro are rare. Recent research has found that mesenchymal stem cells (MSCs) cultured for long time in vitro could lead to malignant transformation, but the mechanism is not very clear. The activation of oncogenes and inactivation of tumor suppressor genes are considered to be one of the mechanisms (6-9). We have found that human embryonic muscle cells which were cultured in vitro long-term could become spontaneously immortalized, and we obtained 6 novel malignant cell lines, this new type of cell lines were named human embryonic muscle-derived malignant transformed cells (hEMTCs). These hEMTCs could be passaged stably and showed characteristics of sarcoma in vivo (10).

Herein, we report several tumor suppressor gene alterations of hEMTCs (from 5 different genetic backgrounds) and we demonstrate homozygous fragment deletions in chromosomal bands 9p21 and extensive mutations of p53 gene.

Materials and methods

Cell culture. Artificial abortive embryos were obtained from Chaozhou Central Hospital, and embryonic use was ratified by the Ethics Committee of Chaozhou Central Hospital, and the informed consent of the mother and/or their family was signed, embryos ranged from 7 to 13 weeks. Leg muscle tissue was cut into 0.5-mm³ pieces, put into 24-well plates, and...
Cultured in Dulbecco’s modified Eagle’s medium (DMEM) (Gibco) containing 10% FCS (Lanzhou Minhai Bio., Co.), 100 μg/ml penicillin, 100 μg/ml streptomycin and 2 μg/ml fluconazole in a 37˚C humidified, 5% CO2 air. When cultured cells became confluent, 0.25% trypsin with 0.02% EDTA was used for passage. After culturing for 4-7 months, the embryonic muscle-derived cells spontaneously transformed in 6 cell lines, and they came from 5 different genetic backgrounds (5 embryos). These hEMTCs could be passaged stably and immortalized. They were named MS0812, MS0504, M3, M4, M5B and M5C, respectively. M5B and M5C were from the same embryo but transformed malignantly in different culture time. The characteristics of line MS0812 was described clearly in our previous report (10). MS0504, M3, M4 and M5C could lead to fibrosarcoma in nude mice. M5B could lead to fibrosarcoma and rhabdomyosarcoma in nude mice which was confirmed by pathology (data not shown). 

**PCR for p16, p15, MTAP and p53.** Genomic DNA was isolated from hEMTCs using a genomic DNA isolation kit (Universal Genomic DNA Extraction Kit Ver. 3.0, Takara Biotechnology Dalian Co., Ltd.) according to the manufacturer’s instructions. The primers for PCR were synthesized in Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. PCR was performed for exons 1-3 of the p16 (CDKN2A), exon 1 of p15 (CDKN2B), exons 2-7 of MTAP (methylthioadenosine phosphorylase), exons 7-8 of p53 in a KP-TC48 thermal cycler (Hybribio Ltd.). PCRs were carried out in 10 mM Tris (pH 8.6), 50 mM KCl, 1.5 mM MgCl2, 0.4% DMSO, 0.2 mM dNTPs, 0.4 mM each primer, 50 ng of genomic DNA, and 5 unit of Taq polymerase (Takara Taq™, Takara Biotechnology Co., Ltd.) per 50-μl reaction. The PCR conditions and primer sequences are shown in Table I. Primers for ß-actin were used as a positive control for the presence of DNA. Electrophoresis of

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**Table I. Sequence of PCR primers.**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequence</th>
<th>PCR conditions</th>
<th>Product size (bp)</th>
</tr>
</thead>
</table>
| **p16 Exon 1** | F: 5’-CGAGAGGGGGGAGACGCGCA-3’  
R: 5’-GGCTGCTACGTTCCCAAATTC-3’ | 95, 60, 72˚C, each for 1 min | 277 |
| **p16 Exon 2** | F: 5’-TTCCTTTCCGTCCATGC-3’  
R: 5’-TGTACAAATCTCAGATCATC-3’ | 95, 57, 72˚C, each for 1 min | 394 |
| **p16 Exon 3** | F: 5’-CCCGCTTCTGATTTTTC-3’  
R: 5’-TTATTTGAGCTTTGCT-3’ | 95, 58, 72˚C, each for 1 min | 355 |
| **p15 Exon 1** | F: 5’-TTTCCCAAAAGGCAATCCCGG-3’  
R: 5’-CGATCTAGGTCCCGCCGATTC-3’ | 95, 59, 72˚C, each for 1 min | 494 |
| **ß-actin** | F: 5’-TGGCACACCACCTCTCATAAGC-3’  
R: 5’-GCACAGCTTCTCCTTAATGTCACG-3’ | 95˚C 1 min, 57˚C 1 min, 72˚C 1.5 min | 341 |
| **MTAP Exon 2** | F: 5’-ATTTGCGAATAATTGGTAGGACGC-3’  
R: 5’-CCAGCAACAAGAAATGAGATGAT-3’ | 95, 57, 72˚C each for 1 min | 396 |
| **MTAP Exon 3** | F: 5’-CAGTCTACATCCAGGTTCCC-3’  
R: 5’-TTCCAAGGGAGGAGCAAT-3’ | 95, 57, 72˚C each for 1 min | 342 |
| **MTAP Exon 4** | F: 5’-CTCTAGAGAGAACACATTGGGT-3’  
R: 5’-GACAGCTACAAATGCTAAGA-3’ | 95, 57, 72˚C each for 1 min | 275 |
| **MTAP Exon 5** | F: 5’-GACCCTAGAAAAAGTTGACT-3’  
R: 5’-TACACCTTCCGAAAGACTA-3’ | 95, 57, 72˚C each for 1 min | 220 |
| **MTAP Exon 6** | F: 5’-AGTGTCATGTGCTATGAT-3’  
R: 5’-ACCAGCTGCATATGTCCTTA-3’ | 95, 57, 72˚C each for 1 min | 328 |
| **MTAP Exon 7** | F: 5’-AGTTCTAGTAACCTCAGTG-3’  
R: 5’-CTACAGACATGCCTGATTG-3’ | 95, 57, 72˚C each for 1 min | 195 |
| **p21 (RT-PCR)** | F: 5’-CAGGGGACACGAGCAGAGGA-3’  
R: 5’-GGGGGCCCCAGGGATGTAC-3’ | 95, 59, 72˚C each for 1 min | 335 |
| **p53 (RT-PCR)** | F: 5’-ACCACCCATCACCACACTAC-3’  
R: 5’-GCAAGCAAGGTTCAAGACG-3’ | 95, 57, 72˚C each for 1 min | 589 |
PCR products was performed on a 2% agarose gel subsequently stained with ethidium bromide and photographed.

**RT-PCR for p21 and p53.** Total RNA was isolated using TRIzol Reagent (Invitrogen) following the recommendations of the manufacturer. cDNA from each cell line was synthesized from denatured RNA (65˚C for 5 min) by incubation at 42˚C for 60 min with final quantities or concentrations of 3 μl of 100 ng/μl oligo (dT), 2 μl of 50 U/μl StrataScript reverse transcriptase, 1 μl of 40 U/μl RNase Ribonuclease inhibitor (Takara, Dalian), and 4 μl 10 mM dNTP mixture. Primers used for p21 and p53 of RT-PCR are summarized in Table I, β-actin was used for RT-PCR control. All RT-PCRs were carried out in a 50X PCR reaction containing 0.4 mM each primer, 5 units of Takara Taq, 1.5 mM MgCl2, 4 μl 10 mM dNTP Mixture. The reaction conditions are shown in Table I.

**DNA sequencing.** The PCR products of exons 7, 8 and intron 7 of p53 and RT-PCR products of p21 were sequenced directly by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (11). The sequence equipment was ABI PRISM 3730. BigDye terminator was used for sequence reagent. All samples were tested by simultaneous bi-directional sequencing (Table II).

**Immunocytochemistry.** MS0812, MS0504, M3, M4, M5B, M5C were cultured in 3.5-cm dishes, and at 80-90% confluence, they were fixed with 4% paraformaldehyde for 15 min, followed by washing in phosphate-buffered saline (PBS) 3 times, each 2 min, endogenous peroxidase activity were quenched in 3% H2O2 for 15 min, then were permeated in 0.25% Triton X-100 for 15 min. The anti-p16 antibody (Zymed, Beijing) was applied at 1:50 dilutions in PBS and incubated in a humidity chamber at room temperature for 2 h.

The secondary antibody staining kit was SP-9000 of broad spectrum provided by the Zhongshan Jinqiao Company, Beijing. AEC served as chromagen. Next, the samples were observed under a powerful optical microscope (x40).

**Results**

Homozygous deletions within chromosome bands 9p21 which included p16, p15 and MTAP. To assess the status of p16 in our cell lines, we used PCR assays in which p16 exons 1-3 fragments were amplified from genomic DNA. No p16 (including exons 1-3) specific product was detected in the 6 hEMTCs. However, specific PCR products using genomic DNA from normal embryonic muscle cells were detected (Fig. 1A-C). p15 and MTAP also locate at chromosome bands 9p21, and their status were assessed, the results showed that p15 exon 1 (Fig. 1D) and exons 3-7 of MTAP (data not shown) were deleted in the 6 cell lines, while the normal control showed specific products (Fig. 1G). Nevertheless, an amplifications of MTAP exon 2 showed several non-specific bands in the 6 hEMTCs, but the positive control of normal embryonic muscle cells showed only one specific band in the same reaction system (Fig. 1E). β-actin was used for internal control and could be detected in all PCRs from the 6 cell lines (Fig. 1F). We performed immunostaining for p16 and found out that the 6 hEMTCs were negative (Fig. 1I), the normal embryonic muscle cells showed positive staining in the cytoplasm (Fig. 1H).
model. Exons 7 and 8 of p53 which compared to the wt p53 in Genebank (http://www.ncbi.nlm.nih.gov/) showed high frequency of mutations which reaching as high as 8% (Fig. 2A) and 14% (Fig. 2B), respectively. All of them were point mutations. The intron 7 changed more significantly, including piece deletion, insertions, frameshift and point mutations, showed almost no similarity to the wt p53 sequence, and we could only use BLAST (Basic Local Alignment Search Tool) for comparing part of the intron 7 sequence (from NT 7174958 to 7174360) (Fig. 2C), and the rest (from NT 7174487 to 7174627) was aligned manually since the structure was changed significantly and BLAST could not be aligned (Fig. 2D).

p53 and p21 of RT-PCR and DNA sequencing of p21. We assayed the transcription of p53 in 6 hEMTCs and only M3 showed the p53 transcription (Fig. 3A). The transcription of p21 in the 6 hEMTCs was also assayed using the RT-PCR. All 6 hEMTCs showed transcription product of p21 but with a non-specific band of about 450 bp in line MS0812, MS0504, M3, M4, M5B and M5C (Fig. 3B), while the normal control showed only one specific band in the same PCR conditions (Fig. 3B). The RT-PCR products of p21 in 6 hEMTCs and normal control were sequenced, and only M3 could be sequenced totally. The expected length was 335 bp, and the real length was 312 bp, the sequence was totally changed and had no similarity to the wt p21 (Fig. 3C), and the sequence signal was specific (Fig. 3D). However, the control (normal human embryonic muscle cells) was the same as wt p21 sequence (data not shown). The other 5 hEMTCs could only be sequenced partly, and the sequenced 180 bp was about the same as corresponding M3 (data not shown). The p21 (RT-PCR) sequence of line M3 (Fig. 3C) exhibited sequential nucleotide ‘T’ and rich ‘GC’ regions, these special structures could lead to the weak sequence signal which make the sequencing impossible. Therefore, we got one complete result.

Discussion

At present, the reports on malignant transformation of cultured human cells in vitro are rare (6-9,12,13), while malignant transformation in vitro could be observed in embryonic cells from mice (14-16). One hypothesis to explain this phenomenon is that the number of mutational events...
Recent research has shown that mesenchymal stem cells can transform malignantly, the mechanism underlying it is not clear yet. It might be associated with the activation of oncogenes and inactivation of tumor suppressor genes, such as the amplification of c-myc and the inactivation of CDK1 family (6,8,14,21). We found that human embryonic muscle cells cultured in vitro could transform malignantly, which suggest that these malignant cells were originated from embryonic muscle cells, and these cell lines could develop sarcoma in nude mice (10). However, malignant transformation mechanism in gene level is not clear yet. The goal of this study was to determine if several classic tumor suppressor genes such as p53, p21 and p16 have an effect on the malignant transformation of embryonic muscle cells.

The interstitial deletions of the 9p21 were observed in a variety of tumor types, including acute lymphoblastic leukemia, glioma, melanoma, lung cancer, head and neck cancer, mesothelioma, ovarian cancer and bladder cancer (22-25). As CDKI family (including p16, p15, and p21) play the predominant role in both p53 and pRb pathways, the inactivation of these tumor suppressor genes in this region, also including MTAP (methylthioadenosine phosphorylase), contributes to the malignant process in all these different tumor types (26,27). CDKN2A generates several transcript variants which differ in their first exons, two of which encode structurally related isoforms known to function as inhibitors of CDK4 kinase (p16). The remaining transcript (CDKN2Aorf; also called p14) is an alternate open reading frame (ARF) that specifies a protein which is structurally unrelated to the

Table II. TP53 mutations of exons 7 and 8 in 6 hEMTCs.

<table>
<thead>
<tr>
<th>Location (codon)</th>
<th>Nucleotide substitution</th>
<th>Mutation effect</th>
<th>Frequency</th>
<th>Mutation recorded</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>231</td>
<td>C -T (ACC -ACT)</td>
<td>Thr (synonymous)</td>
<td>7</td>
<td>No</td>
</tr>
<tr>
<td>235</td>
<td>C -G (AAC -AAG)</td>
<td>Asn to Lys (missense)</td>
<td>0</td>
<td>No</td>
</tr>
<tr>
<td>238</td>
<td>T -C (TGT -TGC)</td>
<td>Cys (synonymous)</td>
<td>4</td>
<td>No</td>
</tr>
<tr>
<td>240</td>
<td>T -C (AGT -AGC)</td>
<td>Ser (synonymous)</td>
<td>9</td>
<td>No</td>
</tr>
<tr>
<td>244</td>
<td>C -G (GGC -GGG)</td>
<td>Gly (synonymous)</td>
<td>21</td>
<td>No</td>
</tr>
<tr>
<td>248</td>
<td>G -C (CGG -CGC)</td>
<td>Arg (synonymous)</td>
<td>3</td>
<td>No</td>
</tr>
<tr>
<td>249</td>
<td>A -C (AGG -CGG)</td>
<td>Arg (synonymous)</td>
<td>6</td>
<td>No</td>
</tr>
<tr>
<td>252</td>
<td>C -T (CTC -CTT)</td>
<td>Leu (synonymous)</td>
<td>8</td>
<td>No</td>
</tr>
<tr>
<td>256</td>
<td>A -G (ACA -ACG)</td>
<td>Thr (synonymous)</td>
<td>4</td>
<td>No</td>
</tr>
<tr>
<td>Exon 8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>262</td>
<td>T -G (GGT -GGG)</td>
<td>Gly (synonymous)</td>
<td>3</td>
<td>No</td>
</tr>
<tr>
<td>264</td>
<td>A -T (CTA -CTT)</td>
<td>Leu (synonymous)</td>
<td>7</td>
<td>No</td>
</tr>
<tr>
<td>268</td>
<td>A -G (AAC -GAC)</td>
<td>Asn to Asp (missense)</td>
<td>0</td>
<td>No</td>
</tr>
<tr>
<td>272</td>
<td>G -T (GTG -GTT)</td>
<td>Val (synonymous)</td>
<td>11</td>
<td>No</td>
</tr>
<tr>
<td>282</td>
<td>G -T (CGG -CGT)</td>
<td>Arg (synonymous)</td>
<td>7</td>
<td>No</td>
</tr>
<tr>
<td>283</td>
<td>C -G (CGC -CGG)</td>
<td>Arg (synonymous)</td>
<td>10</td>
<td>No</td>
</tr>
<tr>
<td>287</td>
<td>G -A (GAG -GAA)</td>
<td>Glu (synonymous)</td>
<td>8</td>
<td>No</td>
</tr>
<tr>
<td>289</td>
<td>C -T (CTC -TTC)</td>
<td>Leu to Phe (synonymous)</td>
<td>4</td>
<td>Infrequent</td>
</tr>
<tr>
<td>291</td>
<td>G -A (AAG -AAA)</td>
<td>Lys (synonymous)</td>
<td>4</td>
<td>No</td>
</tr>
<tr>
<td>293</td>
<td>G -A (GGG -GAA)</td>
<td>Gly to Glu (missense)</td>
<td>0</td>
<td>In skin cancer</td>
</tr>
<tr>
<td>295</td>
<td>C -A (CCT -CAT)</td>
<td>Pro to His (missense)</td>
<td>2</td>
<td>Infrequent</td>
</tr>
<tr>
<td>296</td>
<td>C -T, A -G (CAC -TGC)</td>
<td>His to Cys (missense)</td>
<td>0</td>
<td>In skin cancer</td>
</tr>
<tr>
<td>297</td>
<td>A -C, C -G (CAC -CCG)</td>
<td>His to Pro (missense)</td>
<td>2</td>
<td>In skin cancer</td>
</tr>
<tr>
<td>303</td>
<td>C -T (AGC -AGT)</td>
<td>Ser (synonymous)</td>
<td>2</td>
<td>No</td>
</tr>
<tr>
<td>304</td>
<td>A -G, T -A (ACT -GCA)</td>
<td>Thr to Ala (missense)</td>
<td>4</td>
<td>In skin cancer</td>
</tr>
<tr>
<td>306</td>
<td>C -A (CGA -AGA)</td>
<td>Arg (synonymous)</td>
<td>2</td>
<td>No</td>
</tr>
</tbody>
</table>

Frequency, the mutation frequency on this codon location which has been published. Mutation recorded, this mutation type which has been described in UMD p53 database (http://www.umd.be:2072/).
products of the other variants. Because deletion of the p16
and p14 causes dysregulation of the p53 and pRb pathways
in most cancers, loss of MTAP activity is thought to be
incidental and not of pathogenic consequence (28). However,
in certain cancers, loss of MTAP has been observed in cells
that retain p16. In a study of non-small cell lung cancer,
homozygous deletion of MTAP occurred in 38% (19 of 50)
of the samples compared with only 18% (9 of 50) for p16
(30). The fact that MTAP is lost independently of p16 hints
that loss of MTAP may have some functional basis in tumor
biology (29-31).

We assayed the status of tumor suppressor genes p16, p15
and MTAP which located at 9p21 in the hEMTCs, and all 3
genes were deleted in these cell lines. This suggested that the
interstitial deletion of 9p21 existed in this new type of sarcoma
cell line as reported of adult human spontaneous
transformed stem cells (6). The PCR products of exon 2 of
MTAP showed some non-specific amplification and weak
purpose band, this suggested that there may be a breakpoint
near this location. This result was partly consistent with the
report that a frequent breakpoint took place between MTAP
exon 4 and exon 5 (30).

The p53 tumor suppressor gene is mutated in over 50% of
human cancers, 74% of these mutations are missense,
which result in full-length, albeit mutant, proteins. This fraction
of missense mutations is much higher than in other tumor
suppressor genes (32) and implies that p53 mutant proteins
confer some selective advantage in carcinogenesis. It has
been shown previously that mutant p53 can act as an immor-
talizing gene when cotransfected into primary rat embryo
fibroblasts along with a selectable marker (17), but whether
this protocol works in human embryonic cells is not clear yet.

Human adult spontaneous transformed stem cells were
identified with p53 inactivation by RT-PCR, but detailed
information on p53 was not documented (6). To determine
whether a mutation at the p53 locus is a common event in
the pathways leading to spontaneous cellular immortalization,
6 malignant transformed cell lines were examined for p53
alterations by PCR and DNA sequencing. We checked the
mutations of our cell lines on the UMD TP53 mutation
database (http://www.umd.be:2072/). This is one of the
two most regularly updated databases for TP53 mutations,
the other one is the International Agency for Research on
Cancer (IARC) database (http://www.p53.iarc.fr/index.html/).
Our results were totally different from the p53 mutation database
published (30-33). In particular, the sequence of intron 7 of
p53 in our hEMTCs is completely altered, this is different
from the mutation type of p53 which is mainly point
mutation in malignant cells (37-42). The alterations of p53
codon region will definitely change the transcription and
expression of p53 protein in hEMTCs. The new gain of
function of mutant p53 protein was able to play a role in the
malignant transformation process. All our sequencing
results of p53 (exons 7, 8 and intron 7) in 6 cell lines show
high consistency which suggested that a common mutation
mechanism and strong selective advantage over long-term
culture. The transcription of p53 and p21 in 6 hEMTCs was
also tested using the normal primer sequence (Table I), and
only M3 showed p53 transcription. The negative results in
the other 5 hEMTCs indicated no p53 transcription, the
reason for this was not clear. p53 complicated alterations
may account for this negative result, as indicated by exons 7,
8 and intron 7 sequencing results.

We sequenced all the products (normal control and 6
hEMTCs) and found out that the sequence of the 6 hEMTCs
was aberrant comparing with the wt p21 sequence of normal
control (335 bp, from NT 27510435 to NT 27510573; NT
27511778 to NT 27511973). We obtained one complete
sequence of 312 bp from line M3 in which the sequence
mapping was specific and credible (Fig. 3C and D). The
reason why the transcription of p21 changed so much needs
further research.

In summary, spontaneous hEMTCs in vitro exhibit the
interstitial deletions of the 9p21 which include MTAP, p16
and p15, p53 also changed significantly, the mutation mode
in exons 7, 8 was point mutation, while intron 7 aberration
was complex, including point mutations, fragment deletions
and insertions. Our sequencing results of 6 hEMTCs
(including 5 different genetic backgrounds) showed high
identity in mutation mode, which suggests this mutation mode
is common in our hEMTCs and has a strong selective
advantage over long-term culture. The aberration and inacti-
vation of tumor suppressor genes which include p53, p21,
p16, p14, p15 and MTAP permits DNA-damaged cells
to continue replicating, this frequently contributes to the
accumulation of gene mutations and plays an important role
in the malignant transformation process.

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References
1. Huang PS and Heimbrook DC: Oncogene products as
therapeutic targets for cancer. Curr Opin Oncol 9: 94-100,
1997.
2. Schinzel AC and Hahn WC: Oncogenic transformation and
experimental models of human cancer. Front Biosci 13: 71-84,
2008.
3. Skapek SX, Pan YR and Lee EY: Regulation of cell lineage
specification by the retinoblastoma tumor suppressor. Oncogene
4. Li Y, Nichols MA, Shay JW and Xiong Y: Transcriptional
repression of the D-type cyclin-dependent kinase inhibitor p16
by the retinoblastoma susceptibility gene product pRb. Cancer
6. Rubio D, Garcia S, Paz MF, De la Cueva T, Lopez-Fernandez
LA, Lloyd AC, Garcia-Castro J and Bernad A: Molecular
characterization of spontaneous mesenchymal stem cell
and Zhang X: A novel tumor cell line cloned from mutated
human embryonic bone marrow mesenchymal stem cells.
8. Rubio D, Garcia-Castro J, Martin MC, de la Fuente R, Cigudosa JC,
Lloyd AC and Bernad A: Spontaneous human adult stem cell
9. Gibbs CP, Kukkoken VG, Reith JD, Tchigrinova O, Suslov ON,
Scott EW, Ghivizzani SC, Ignatova TN and Steindler DA:
Stem-like cells in bone sarcomas: implications for tumor
line with characteristics of dedifferentiated chondrosarcoma.


