Transformation of Late Passage Insulin-Like Growth Factor-I Receptor Null Mouse Embryo Fibroblasts by SV40 T Antigen

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Abstract

There is evidence that the insulin-like growth factor-I (IGF-I) receptor is required for transformation by a variety of viral and cellular oncogenes and overexpressed growth factor receptors in a mouse embryo fibroblast model. To further investigate the IGF-I receptor signaling pathways that are required for the permissive effect of the receptor on transformation by SV40 T antigen, we established three independent fibroblast cell lines each from wild-type and IGF-I receptor null embryos (R<sup>−</sup>). We transfected the wild-type and R<sup>−</sup> cell lines with an SV40 T antigen plasmid and selected three clones from each cell line that expressed T antigen. As in previous reports, none of the cloned R<sup>−</sup> cell lines expressing T antigen were transformed as measured by the ability to form large colonies in soft agar. However, with further passage, all three T antigen–expressing clones from one of the R<sup>−</sup> cell lines (R<sup>3</sup>) formed large colonies in soft agar and the transformation of these T antigen–expressing clones was confirmed by tumorigenesis experiments in immunodeficient mice. DNA microarray analysis comparing gene expression between early passage and late passage R<sup>3</sup>/T antigen clones showed, among other changes, an increase in the expression of ErbB-3 mRNA in the late passage clones. Also, the expression of ErbB-3 protein was dramatically increased in the late passage R<sup>3</sup>/T antigen clones. We conclude that late passage IGF-I receptor null mouse embryo fibroblasts can be transformed by SV40 T antigen, and that ErbB-3 may play a role in permitting transformation by T antigen. (Cancer Res 2006; 66(8): 4233-9)

Introduction

Over the past decade, evidence has accumulated that insulin-like growth factors (IGF-I and IGF-II) play an important role in cancer development and progression. High blood IGF-I levels are a risk factor for development of cancer of the breast, prostate, colon, and lung (1). Cell lines from a variety of human cancers have been shown to be responsive to IGFs in monolayer culture (2, 3). The IGF-I receptor and IGF-II have been shown to be overexpressed in some cancers (4). There is genetic evidence that the M6P/IGF-II receptor, which provides a degradative pathway for IGF-II, is required for transformation by various viral and cellular oncogenes. However, Grb2 (24) in R<sup>−</sup>/C0 came from the observations that overexpression of IRS-1 (23) or Grb2 (24) in R<sup>−</sup>/T antigen cells restored transformation.

Materials and Methods

Establishment of cell lines from Igf1r<sup>−/+</sup>, Igf1r<sup>−/−</sup>, and IGF1r<sup>−/−</sup> embryos. IGF1r<sup>−/−</sup> mice (MF1/129) were kindly provided by A. Efstatiadis (Department of Genetics and Development, Columbia
University, New York, NY) to A. Dlugosz for mating (25). Four normal-sized and four small embryos were selected from a litter. Gestational age was estimated to be 16 days based on crown-rump measurements of the normal size embryos. The 3T3 method of Todaro and Green (26) was adapted for establishing cell lines. Mined embryos were disaggregated with 0.2% trypsin. Cells were plated in high-glucose DMEM containing 2,200 mg/L of sodium bicarbonate and 10% FCS (growth medium) at a density of 5 × 10^5/60 mm dish and incubated at 37°C in an atmosphere of 5% CO₂.

After 3 days of growth, monolayers were trypsinized and subcultured at a density of 3 × 10^5/60 mm dish. This subculturing protocol (3T3) was repeated every 3 days until cell lines were established as assessed by increased proliferation rate or ability to proliferate after plating at low density. The WT and R™ cell lines were not single cell cloned.

**Genotyping.** DNA was purified from MEF cell lines (Qiagen DNA Mini Kit) and PCR amplification was done using primers which flank exon 3 of the mouse Igfr gene (primer 1, ATCACCTTACACCCCT; primer 2, GGACCCTTCAAGGTITTAG). The WT allele generates a 1,536 bp product. The PCR assay was also done with three primer combinations (27) to confirm the genotype. A 431 bp product is generated.

**Binding of [3H]-long R™IGF-I to MEFs.** Binding of the IGF-I analogue, long if IGF-I, to monolayer cultures of WT and R™ cells was done as described by Siebler et al. (28).

**DNA synthesis.** DNA synthesis in the MEF lines was measured using a bromodeoxyuridine (BrdUrd) immunoocytochemical/histochemical assay (5-bromo-2′-deoxyuridine labeling and detection kit II, Roche Molecular Biochemicals, Mannheim, Germany). Cells were plated in growth medium in LABTEK II chamber slides (PGC Scientific, Frederick, MD) at a density of 500 cells per well, and 24 hours later, medium was changed to serum-free media.

**Transfection of WT and R™ cells with SV40 large T antigen.** The WT and R™ cell lines were transfected with pRSV-neo vector and pRSV-neo vector together with pCDNA6/V5-His (blasticidin selection marker) using Lipofectamine Max (GibcoBRL, Gaithersburg, MD) in an atmosphere of 5% CO₂. The MEF lines used for transfection were WT/2-P26, WT/3-P36, WT/4-P23, R™-2-P21, R™-3-P25, and R™-4-P38. Clones were obtained from cell cultures by limited dilution. Clones were screened for T antigen expression by immunoblotting with SV40 T antigen monoclonal antibody (CalBiochem Immunochemicals, San Diego, CA) after SDS-PAGE of cell lysates and transfer of protein bands to nitrocellulose membrane. Three clones of T antigen and empty vector transfectants were chosen for each of the WT and R™ cell lines.

**Microarray analysis.** A DNA microarray described by Shaffer et al. (31) was used to compare three clones of early passage R™/T antigen cells with three clones of late passage R™/T antigen cells for gene expression. Gene expression was also compared in three clones of WT/3/vector cells and three clones of WT/3/T antigen cells in order to screen out genes which changed as a result of transformation by T antigen. RNA was prepared from cells growing in log phase using the RNeasy kit from Qiagen. Experimental sample RNAs were converted to Cy5-labeled cDNA and cohybridized on DNA microchips with RNA from a reference pool labeled with Cy3. The reference pool is a mixture of total RNA from 20 cell lines, representing many cell lineages, which serves as a common denominator against which all samples may be compared. After determining the signal intensity for each element by confocal laser microscopy using a Genepix scanner (Molecular Devices, Sunnyvale, CA), gene expression was expressed as the ratio of the experimental sample signal divided by the reference pool signal (Cy5/Cy3). For each pair of age, only those genes whose signal variance over control when both were considered for subsequent analysis. Hierarchical clustering was done using the Cluster and Treeview programs (32). Comparisons between the early and late R™/T antigen clones were expressed as fold differences and only genes for which fold differences were >1.5 were reported. Statistical analyses of the comparisons used Student’s t test and P < 0.05 were considered significant.

**Results**

**Establishment of WT and IGF-I receptor null fibroblast lines.** Heterozygous IGF-I receptor knockout mice (25) were mated. At 16 days of gestation, some of the embryos were only 1/2 to 2/3 the size of other embryos in the litter. These small embryos were assumed to be Igfr(−/−), whereas the larger embryos were assumed to be either Igfr(+/+) or Igfr(+/-). To establish cell lines from WT and R™ embryos, the 3T3 protocol of Todaro and Green (26) was adapted. Cells were trypsinized and counted every third day and 3 × 10^5 cells were freshly plated on a 60 mm dish.

The prototypic proliferation pattern during the process of establishment of MEF cell lines is that the proliferation rate over a 3-day period initially is high and then declines to 0 after approximately 10 passages (crisis; ref. 26). Proliferation rate then gradually increases, and at this point, a cell line has been established. We were able to establish cell lines from three WT and three
R'/embryos. Although fibroblasts from three of the embryos (WT2, R' 2, and R' 3) exhibited a classical proliferation pattern during the process of establishment, cells from the other three embryos (WT3, WT4, and R' 4) did not show a dramatic increase in proliferation in the later passages. This was because these three lines exhibited contact inhibition under the conditions of the 3T3 protocol. When these lines were plated at low density, they proliferated at a rate characteristic of established MEF cell lines. Although the proliferation pattern seen in establishing cell lines according to the 3T3 protocol was variable among the six cell lines, there was no pattern which distinguished the WT from the R' cell lines. The absence of the IGF-I receptor did not confer a decrease in average proliferation rate during precrisis passages 3 to 6 (N/No = 3.42 ± 0.65 for WT versus 3.39 ± 0.53 for R'). Uniform differences in morphology between the WT and R' cell lines were not observed. However, at a higher density, the R' 2 and R' 4 cells exhibited a wide, flat, and translucent morphology (data not shown).

**Genotyping of the MEF lines.** Genotyping of the putative WT and R' cell lines was done by PCR amplification of genomic DNA using primers which flank exon 3, which had been replaced with the neo cassette to produce the IGF-I receptor null allele (ref. 25; Fig. 1). The WT allele generates a 536 bp product and the knockout allele generates a 1,336 bp product. Only the 1,336 bp product was found in the three R' cell lines confirming the Igfr (-/-) genotype, whereas only the 536 bp product was found in two of the WT cell lines, indicating an Igfr (+/+) genotype. Both products were found in the WT3 cell line indicating an Igfr (+/-) genotype. These results were confirmed by PCR amplification of genomic DNA using primers within the neo cassette (27). The expected 431 bp fragment was generated from the R' cells and the WT3 cell line (data not shown).

**Ligand binding to the IGF-I receptor.** IGF-I receptor expression in the WT and R' cells was examined by performing ligand binding studies with a radiolabeled analogue of IGF-I (\[125I\]-longR3IGF-I) that binds poorly to IGF binding proteins (28, 33). Specific binding of IGF-I was not detected in the R' cell lines. Scatchard analysis of longR3IGF-I binding showed that receptor number ranged from 28,000 to 56,000 per cell in the three WT cell lines; mean Ks values ranged from 5.0 x 10^5 mol/L^-1 to 7.4 x 10^5 mol/L^-1.

**Cell proliferation and DNA synthesis.** The doubling times of the WT and R' cell lines in medium containing 10% FCS were not significantly different (23.7 ± 7.3 hours for WT versus 24.9 ± 5.7 hours for R'). Neither WT or R' cells proliferated in defined medium containing EGF, PDGF, and IGF-I. However, when DNA synthesis was measured by nuclear labeling with BrdUrd, WT cells showed significant increases in the percentage of cells labeled with BrdUrd to 35% to 65% in response to increasing amounts of IGF-I added to a mixture of PDGF and EGF at fixed concentrations, whereas the R' cell lines showed no significant increases in labeling index with the addition of IGF-I (data not shown).

**Transfection of WT and R' cell lines with SV40 T antigen.** We transfected WT and R' cell lines with a PRSVneo vector containing the SV40 early region encoding large and small T antigens (34). Transfectants were single cell cloned and then tested for T antigen expression by immunoblotting cell lysates after SDS-PAGE (data not shown). Three T antigen–transfected and three empty vector–transfected clones were chosen from each of the WT and R' cell lines. The number of large colonies (>100 μm) in soft agar after 2 weeks was used as a measure of transformation (Fig. 2a and b). T antigen transfectants of two of the WT cell lines formed large colonies in soft agar, but T antigen transfectants of the WT2 cell line did not form large colonies. We conclude that the presence of the IGF-I receptor is not sufficient for transformation of the WT2 cells by T antigen. Initially, none of the T antigen transfectants of the R' cell lines formed large colonies in agreement with the report of Sell et al. (11). However, later passages of all three clones of the R' 3/T antigen transfectants did form large colonies in soft agar. It is likely that the ability of these R' 3/T antigen transfectants to form large colonies is dependent on the presence of T antigen because the three empty vector clones for the R' cell line did not form large colonies even at late passages.

The results of the soft agar assay were confirmed by a more stringent test of transformation, tumorigenesis in nude mice (Fig. 2c and d). The WT3 T antigen clones and the late passage R' 3 T antigen clones produced fibrosarcomas in nude mice, whereas the vector-only clones did not produce tumors (WT3) or produced tumors which appeared only after 65 days (R' 3).

**Expression of IRS-1, Grb2, and phospho-FAK in early and late passage R' 3/T antigen cells.** Overexpression of IRS-1 in R' cells expressing T antigen resulted in transformation (23). Similarly, overexpression of Grb2 in R'/T antigen cells resulted in transformation as measured by colony formation in soft agar (24). R' cells could be transformed by v-src but not by activated c-src (13). The v-src transformed R' cells exhibited higher levels of tyrosine phosphorylation of FAK. These observations prompted us to examine the expression of IRS-1, Grb2, and phospho-FAK in early and late passage R' 3/T antigen cells (Fig. 3). IRS-1 expression was not increased in the late passage cells. Increase in Grb2 was not a uniform finding among the three late passage clones. The level of phosphorylated FAK was examined by a combination of immunoprecipitation with anti-FAK beads and immunoblotting with anti-pTyr and found not to be increased in the late passage cells. In addition, the level of phosphorylation on specific tyrosine residues (Tyr397 and Tyr576) of FAK was not increased in the late passage cells. Thus, transformation of late passage R' cells with T antigen could not be explained by increased expression of IRS-1, Grb2, or phospho-FAK.

**Microarray analysis of gene expression in early and late passage R' 3/T antigen cells.** To further identify changes in late passage R' 3/T antigen cells that led to transformation by T antigen, we compared the three clones of early passage R' 3/T antigen cells with the three clones of late passage R' 3/T antigen cells for gene expression. The results are shown in Tables 1 and 2.
and Fig. S1 and File 1 in Supplementary Data. In Tables 1 and 2, we eliminated genes whose differences in expression were a result of (rather than a prelude to) T antigen transformation, as determined by comparison of gene expression in three clones of WT3/vector and three clones of WT3/T antigen cells (Fig. S1 and File 1 in Supplementary Data). Several of the genes that were expressed at higher levels in late passage R^+/C0^3/T antigen clones are important in cell cycle control or tumorigenesis: fibroblast-activating protein (Fap), Erbb3, Nck1, and frizzled (35–38). We were able to examine the expression of ErbB-3 protein and Nck1 protein in cells in log phase growth by immunoblotting of cell extracts. There was a dramatic increase in the expression of ErbB-3 in the late passage R^+/C0^3/T antigen clones (Fig. 4). There was no difference in the expression of Nck1 between the early and late R^+/C0^3/T antigen clones (data not shown). In the list of genes that were expressed at lower levels in the late passage R^+/C0^3/T antigen cells, Apc and Ctgf have been shown to play roles in tumorigenesis, whereas lats2 is a putative tumor suppressor (39–43). We were able to examine the expression of APC protein by immunoblotting and found no difference in the level of expression between early and late passage R^+/C0^3/T antigen cells in log phase growth (data not shown). CTGF was not detected in either the early or late passage R^+/C0^3/T antigen cells.

Discussion

In the current study, we have sought to further examine the findings of the Baserga laboratory (11), that the IGF-I receptor must be present for transfection of MEFs by SV40 T antigen. We established three new R^+/C0^ cell lines from the IGF-I receptor knockout embryos. In contrast to the results of Sell et al. (12), we found that the doubling time of precrisis R^+/C0^ cells in 10% FCS (21.2 hours) was not significantly different from WT cells (21.1 hours). Sell et al. (12) reported that the doubling time of precrisis R^+/C0^ cells was 109 hours and the doubling time of WT cells was 43.6 hours and that all phases of the cell cycle were elongated in the R^+/C0^ cells. The increased doubling times for both R^+/C0^ and WT cells compared with our data suggest that Sell et al. (12) studied cells at a later time in the precrisis period. In addition, the R^+/C0^ cells studied by Sell et al. (12) were derived from IGF1R^+/−/IGF2^+/−/embryos (44).

Figure 2. A and B, colony formation in soft agar for SV40 T antigen transfectants of WT and R^−/C0^ cells. Cells (3 × 10^4) were plated in 35 mm dishes. Colonies >100 μm were counted after 2 weeks. Vector transfectants (V) and T antigen transfectants (T) for the three WT cell lines in (A) and the three R^−/C0^ cell lines in (B). For the R^−/C0^/T antigen cells and R^−/C0^/vector results for early (E) and late (L) passages are shown. These early passage and late passage cells are defined in Materials and Methods. Experiments were repeated two to four times and combined for statistical analysis. **, P < 0.01, T versus V clones; *, P < 0.05, T versus V clones. C and D, tumorigenesis in nude mice following injection of clones of WT3/T antigen (T) and empty vector (V) transfectants (C) and injection of clones of late passage (L) R^−/C0^/T antigen (T) and empty vector (V) transfectants (D). Points, mean for five mice; bars, SE. Tumor weight is plotted versus days after injection.
whereas our R^{-} cells were from \textit{Igf1r}(-/-) embryos. However, it is unlikely that IGF-II produced by \textit{Igf1r}(-/-) cells would contribute significantly to proliferation via activation of the insulin receptor in a background of 10% FCS which contains IGF-II. In addition, Morrone et al. (45) found that R^{-} cells showed only a 10% increase in cell number in response to 100 ng/mL of IGF-II.

Whereas Baserga's group transfected a single WT and a single R^{-} cell line with tsA58Tag plasmid or a WT T antigen plasmid, and presented data from a single clone of each type, we transfected the three WT cell lines and the three R^{-} cell lines with WT SV40 T antigen and selected three clones for each cell line. Both Baserga's constructs and ours carried genomic SV40 early region DNA which contains the overlapping genes for large, small, and 17K T antigens (46). We tested the clones for anchorage-independent growth as a measure of transformation. None of the T antigen–expressing clones from the WT2 cell line formed large colonies in the soft agar assay. This was not because of a low level of expression of T antigen in the WT2 cell line because T antigen was expressed at a lower level in the WT3 clones, which did exhibit a transformed phenotype. Also, the WT2 cell line was homozygous for \textit{Igf1r} and exhibited a higher level of 125I-IGF-I binding than the WT3 and WT4 cell lines. In agreement with the report of Sell et al. (11), we initially found that none of the T antigen clones from the R^{-} cell lines were transformed as assessed in the soft agar assay. However, later passage cells from each of the T antigen clones from the R^{-} 3 cell line did form large colonies in the soft agar assay. Transformation of these late passage R^{-} 3 clones was attributed to the presence of T antigen because the three late passage empty vector clones from the R^{-} 3 cell line did not form colonies in soft agar. The transformed phenotype of the late passage R^{-} 3 T antigen clones was confirmed by tumorigenesis studies in immunodeficient mice. The three clones of the WT3/T antigen cells and the three clones of the late passage R^{-} 3/T antigen cells produced tumors, whereas empty vector clones did not produce tumors (WT3) or produced tumors much later (R^{-} 3). We conclude that with further passage, the R^{-} 3 T antigen clones changed so that the cells could become transformed by T antigen. We propose that this change(s) effectively substituted for the role of the IGF-I receptor in permitting transformation by T antigen. All three of the R^{-} 3 T antigen clones changed, suggesting that the change depended on an intrinsic property of the R^{-} 3 cell line (perhaps related to its less flattened morphology) rather than being a stochastic event. We expect that there are both baseline differences in gene expression in R^{-} 3 cells (as opposed to R^{-} 2 and R^{-} 4 cells) and acquired changes in late passage R^{-} 3 cells (as opposed to early passage R^{-} 3 cells) that contribute to the ability to become transformed by SV40 T antigen.

In many systems, it has been shown that extended passage of cultured cells could result in multiple genetic changes which increase susceptibility to transformation by cooperating oncogenes or which may even cause spontaneous transformation. For example, Kunisada et al. (47) reported that late passage rat embryo fibroblasts or cells taken from adult rats were more easily transformed by SV40

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<tr>
<th>Gene</th>
<th>Fold</th>
<th>P</th>
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<tbody>
<tr>
<td>Siat8d, sialyltransferase 8</td>
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<td>0.049</td>
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<tr>
<td>Siat8d, sialyltransferase 8</td>
<td>1.30</td>
<td>0.017</td>
</tr>
<tr>
<td>Mi1, metallothionein 2</td>
<td>2.97</td>
<td>0.052</td>
</tr>
<tr>
<td>Pnicap, peptidylprolyl isomerase C-associated protein</td>
<td>1.57</td>
<td>0.047</td>
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<tr>
<td>mouse thyosin cDNA, AK017910</td>
<td>1.58</td>
<td>0.027</td>
</tr>
<tr>
<td>Mi1, metallothionein 2</td>
<td>2.54</td>
<td>0.007</td>
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<tr>
<td>Fap, fibroblast activation protein</td>
<td>1.43</td>
<td>0.043</td>
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NOTE: Early passage and late passage R^{-} 3/T antigen clones are defined in Materials and Methods.

**Table 1. Genes that are expressed at a higher level in late passage R^{-} 3/T antigen clones than in early passage R^{-} 3/T antigen clones**

**Figure 3.** Expression of IRS-1, Grb2, and phospho-FAK in early and late passage R^{-} 3/T antigen cells. Extracts (30 µg protein) or α-FAK immunoprecipitates of extracts from three early passage and three late passage clones of R^{-} 3/T antigen cells were analyzed by SDS-PAGE and immunoblotting was done with the indicated antibodies.
DNA than early passage fibroblasts or cells from young animals. Schiller and Bittner (48) found that human bronchial epithelial cells transfected with SV40 T antigen became tumorigenic at late passage in vitro, a change that included expression of α5/β1-integrin, and that could be further selected for by passage in athymic mice. More recently, Zhao et al. (49) reported that whereas early passage human mammary epithelial cells expressing human TERT (HMECs-hTERT) required expression of H-ras V12 for transformation by SV40 early region, late passage HMECs-hTERT could be transformed using SV40 early region alone. Here, we show that further passage of a IGF-I receptor null fibroblast line, has rendered the cells permissive for transformation by the SV40 early region.

The role of the IGF-I receptor in spontaneous transformation of MEFs has not been studied. However, tumorigenesis experiments showed that the late passage R-3/vector clone produced tumors in the immunodeficient mice if the experiment was carried out to 65 days, and this result suggests that the IGF-I receptor may not be required for spontaneous transformation. Other examples of IGF-I receptor–independent transformation are transformation by v-src (13) and transformation by a GTPase-deficient mutant of human Gα13 (50).

Table 2. Genes that are expressed at a higher level in early passage R-3/T antigen clones than in late passage R-3/T antigen clones

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<td>4.04</td>
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<td>Ctgf, connective tissue growth factor</td>
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<td>2.39</td>
<td>0.041</td>
<td>Csf1, colony stimulating factor 1</td>
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<td>1.97</td>
<td>0.042</td>
<td>Akap12, A kinase (PRKA) anchor protein</td>
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<td>1.87</td>
<td>0.028</td>
<td>Stab2, stabulin 2</td>
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<tr>
<td>1.79</td>
<td>0.045</td>
<td>Akap12, A kinase (PRKA) anchor protein</td>
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<td>Tpr1, tyrosinase-related protein 1</td>
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<td>Wdr 1, WD40 repeat</td>
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<td>Polr2b, RNA pol II B</td>
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<td>Mhf fd2, methylenetetrahydrofolate dehydrogenase</td>
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<td>0.034</td>
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<td>1.62</td>
<td>0.013</td>
<td>Afl5, activating transcription factor 5</td>
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<tr>
<td>1.61</td>
<td>0.016</td>
<td>Hspa9a, heat shock protein, A</td>
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<td>Eif1a, eukaryotic translation initiation factor 1A</td>
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<td>0.027</td>
<td>Cskn1a1, casine kinase 1, α1</td>
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<td>0.027</td>
<td>Mfhd2, methylenetetrahydrofolate dehydrogenase</td>
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<td>Nrd1, nuclear receptor subfamily 1, group D, member 1</td>
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<td>lats2, large tumor suppressor 2</td>
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<td>1.53</td>
<td>0.031</td>
<td>Hspa9a, heat shock protein, A</td>
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<td>U3 snRP 1.53 0.041 sphingomyelin phosphodiesterase</td>
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<td>1.53</td>
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<td>Ate1, arginine-tRNA-protein transferase 1</td>
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<td>1.52</td>
<td>0.044</td>
<td>Ape, adenomatous polyposis coli</td>
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NOTE: Early passage and late passage R-3/T antigen clones are defined in Materials and Methods.

Figure 4. Expression of ErbB-3 in early and late passage R-3/T antigen cells. Extracts (30 μg of protein) from three early passage and three late passage clones of R-3/T antigen cells were analyzed by SDS-PAGE and immunoblotting was done with an ErbB-3 antibody. The identity of the ErbB-3 band was confirmed by immunoblotting in the presence of a peptide which served as antigen in the development of the ErbB-3 antibody (data not shown).

To identify the changes in late passage R-3/T antigen cells that might permit transformation by T antigen, we analyzed gene expression by microarray analysis in early and late passage R-3/T antigen clones. ErbB-3 gene expression was increased significantly in late passage R-3/T antigen cells. Moreover, the expression of ErbB-3 protein was dramatically increased in the late passage cells. ErbB-3 is an impaired kinase due to substitutions in critical residues in its catalytic domain, and is only capable of signaling as a receptor heterodimer (36). The favorite partner for ErbB-3 is ErbB-2, which lacks an activating ligand. The ErbB-3/ErbB-2 dimer is the most potent module of the ErbB receptor family for signaling proliferation and transformation. This is due in part to the presence of multiple phosphotyrosine residues in the carboxy-terminus of ErbB-3, which are part of motifs that engage the p85 regulatory subunit of phosphoinositide-3-kinase, leading to activation of the Akt pathway. In addition, ErbB-2 enhances the affinity of the ErbB-3/ErbB-2 heterodimer for ligands and broadens the list of ligands that bind to the heterodimer. Also, down-regulation of the ErbB-2/ErbB-3 receptor pair by endocytosis is slower than for ErbB-1-containing pairs resulting in slower degradation of ligand and receptor. Although overexpression of ErbB-1 (EGF receptor) in R-3/T antigen cells did not result in transformation (18), it is possible that the ErbB-2/ErbB-3 heterodimer could activate other pathways that would permit transformation by T antigen.

The microarray data in Supplemental Fig. S1 shows that the level of ErbB-3 in the early passage R-3/T antigen clones is higher than the level in WT3/vector clones, suggesting that this relatively high basal level of ErbB-3 may be a distinguishing characteristic of the R-3 cell line. Further increase in ErbB-3 levels in the late passage R-3/T antigen clones may contribute to transformation by SV40 T antigen.

We conclude that late passage IGF-I receptor null MEFs can be transformed by SV40 T antigen. Our finding of increased expression of ErbB-3 mRNA and protein in late passage R-3/T antigen cells raises the possibility that signaling by the ErbB-3/ErbB-2 heterodimeric receptor could substitute for IGF-I receptor signaling in permitting transformation of MEFs by SV40 T antigen. This does not preclude possible additional contributions due to the intrinsic properties of the R-3 cell line or other changes in the R-3 cell line or its later passage T antigen–transfected derivatives.

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References

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