Cancer cell lines' growth is promoted through individual responsiveness to autocrine and/or exogenous erythropoietin in vitro

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Abstract: Erythropoietin (Epo) therapy for combatting anemia or fatigue in cancer patients has become a controversial issue. We have previously reported our study of 24 malignant human cell lines which express Epo and its receptor (EpoR) mRNAs and secrete Epo protein; blockade of Epo-signal destroyed the xenografts of female malignancies [1] and cancer cell lines [2]. We speculated that the conflicting clinical outcomes are due to the individual responsiveness to Epo of the various cancers. We measured the expression levels of Epo and EpoR mRNAs and the amount of Epo protein secreted and demonstrated the presence of EpoR protein in these 24 cell lines, some of which had anoxia-inducible Epo and/or EpoR mRNA. Additionally in seven selected cell lines with known amounts of Epo and EpoR expression, rhEpo triggered and the EpoR antagonist (EMP9) inhibited tyrosine phosphorylation of STAT5. They showed a rhEpo-induced growth that corresponded generally to the level of the constitutive activation of tyrosine phosphorylation of STAT5. Further, EMP9-suppressed growth depended inversely on the amount of Epo secretion. These data justify our speculation that the growth of very many cancers is promoted by their own Epo-signal which may or may not be accelerated by exogenous rhEpo.

Key-Words: Erythropoietin; Erythropoietin receptor; Malignant cell lines; Signal transduction; Epo assay; IdU assay; Real-time RT-PCR analysis; Western blotting

1 Introduction

Erythropoietin (Epo) is a cytokine involved in the regulation of red blood cell production [3]. Epo binds to its receptor (EpoR) on erythroid progenitors, induces receptor homodimerization and subsequent activation of JAK2 through tyrosine phosphorylation leading to activation of STAT5 that induces mitosis [4,5] and has an anti-apoptotic function [6] in erythroid progenitors. Simultaneously, the volume of circulating

erythrocytes is rigidly controlled not only by the number of EpoR sites on a developing erythroblast [7], but also by endogenous Epo production in developing erythroids at the peak proliferation stage leading to their own growth in an autocrine manner [8-11]. Moreover, Epo mRNA is under the control of hypoxia-inducible transcription factor (HIF-1) [12,13].

Since we first reported that normal [14] and malignant female reproductive organs [15] express Epo and EpoR mRNA, there has been increasing evidence of the involvement of the Epo-EpoR pathway not only in normal physiological functions but also in the promotion of very many cancers [16]. Moreover, deprival of Epo by Epo antibody, the soluble form of EpoR or EpoR antagonist, EMP9 [17], destroyed xenografts of female reproductive organ malignancies [1] and malignant cell lines [2] resulting in the death of malignant cells and their accompanying microvessels.

Two recent clinical rhEpo trials one in a group of non-anemic breast cancer patients [18] and one in a group of anemic head-and-neck cancer patients [19] failed to improve their anemia, survival or quality of life. One of the problems noted in these two clinical projects and various preclinical and clinical reports on the results of rhEpo treatment, effective or not, is that it has been shown that the expression of EpoR is functionally active in these tumors. If so, the detecting protein, anti-EpoR antibody, is specific for EpoR [20]. These concerns will be resolved if it can be determined how various cancers respond to Epo through an in vitro analysis of how individual malignant cells are involved in the Epo-signal.

Autocrine secretion, the ability of malignant cells to produce and to respond to their own growth was seen in 24 malignant human cell lines [2] which express both Epo and EpoR mRNAs and secrete Epo protein regardless of their origins, types, genetic characteristics or biological properties. We then analyzed these cell lines in vitro to show how various types of cancer proliferate through autocrine and/or exogenous Epo in selected seven cancer cell lines with various levels of Epo and EpoR mRNA and of Epo and EpoR protein, and examined the responsiveness of these cell lines to rhEpo and EMP9 in vitro. We report here that individual expression levels of the Epo and EpoR transcripts and proteins in the 24 cell lines, and the individual levels of constitutive activation of tyrosine phosphorylation of STAT5 corresponded generally to the growth activity of the selected cell lines. Moreover, these cell lines exhibited rhEpo-triggered and EMP9-suppressed tyrosine phosphorylation concomitant with rhEpo-induced and EMP9-reduced growth. These results may explain inconsistent clinical outcomes, and suggest that we should avoid giving a dire prognosis whenever Epo-signal is identified in a malignant biopsy.

2 Materials and Methods

2.1 Cell cultures and culture conditions.

The human cell lines used and their developmental origins are listed in Table 1. The culture media and conditions under normoxia and anoxia were described previously [2]. All culture media without ribo-, and deoxyribo-nucleosides are products of Gibco ERL (NY, U.S.A.), and are usually supplemented with 10% fetal bovine serum (FBS; SAFC Bio Science, Brooklyn, Victoria, Australia).

2.2 Real-time quantitative PCR analysis.

Total RNA was isolated from each cell line with TRIzol reagent (Invitrogen, Carlsbad, CA, U.S.A.), and cDNA was synthesized by AMV reverse transcriptase XL (Takara Bio, Otsu, Japan) and was amplified with the Platinum q PCR SuperMix-UDG (Invitrogen) and respective Epo (Hs 00171267-m1),

EpoR (Hs 00181092-m1) and 18S rRNA (TaqMan Ribosomal RNA Control Reagents, 4308329) primer and probe (Applied Biosystems, Foster, CA, U.S.A.).

2.3 Epo assay.

Cells cultured in each medium without FBS for 24 h under normoxia or anoxia were used for the assay. One ml of 10-fold-concentrated culture medium containing 1×10^7 cells per 10 ml of each cell line was used. Epo protein was measured in triplicate with an enzyme-linked immunoassay as described previously [2]. The protein content of each sample was determined in all cells in a culture dish with a protein assay kit (Bio Rad, Hercules, CA).

2.4 Western blots and immunoprecipitation.

In each cell line, 1×10^7 cells under normoxia and four cell lines in which EpoR mRNA was raised under anoxia were lysed, and the membrane fraction was processed for western blotting as described previously [2]. Polyclonal anti-EpoR antibody (C-20, Santa Cruz, CA, U.S.A. Lot#D232) and anti-N-terminal EpoR antiserum [21] were used.

2.5 Signal transduction assay.

After the seven cell lines had been cultured in serum free medium for 24 h, they were treated with 5 U/ml of rhEpo for 5 min, or 0.25 or 0.5 mg/ml of EMP9 three times at 5 min intervals for SCH and 15 min intervals for others or consecutive treatment with EMP9 and rhEpo. After treatment the cells were processed for immunoprecipitation by incubation with polyclonal anti-STAT5 antibody (H134, Santa Cruz, CA, U.S.A.) at 4°C overnight. Immunocomplexes were collected as described previously [2], processed for western blotting, and caused to react with anti-phosphotyrosine (Upstate, Temecula, CA, U.S.A.) [2]. The results were analyzed when three identical data were obtained in five independent experiments. The band intensity was quantified with Image J, in the public domain NIH image program (http://rsb.info.nih.gov/ij/).

2.6 Proliferation assay.

rhEpo was a gift of the Life Science Institute of Snow Brand Milk Product Co. (Tochigi, Japan). Cells were incubated for four days in a concentration of 4×10^2 cells in 200 μl of medium containing 0, 2.5, 5, 10 or 25 U/ml of rhEpo which was changed every day. EMP9 was synthesized by Peptide Institute (Minoo, Osaka, Japan). Cells were cultured in a concentration of 1×10^3 cells in 200 μl containing 0, 0.1, 0.25, 0.5 or 1.0 mg/ml of EMP9, which was changed four times at 1 h intervals, then incubated for 24 h. After both treatments, each growth was determined by colorimetric IdU assay (MK-420, Takara, Japan).

2.7 Statistics.

Quantitative data were expressed as mean ± SEM from at least 3 experiments and compared by Student's t-test. P values less than 0.05 were considered significant.

3 Results

3.1 Expression of Epo in malignant cell lines.

The amount of Epo mRNA in 24 malignant cell lines ranged from 1.95 ± 0.92 in G361 to 258.36 ± 102.67 in SCH under normoxia (Fig. 1A). Since the oxygen content of cells in malignant human tumor xenografts located 200 μm from the nearest capillary is reported to be 0.2% leading to acquisition of hypoxia-resistance [22] which upregulates Epo mRNA [23], we exposed the cell lines under anoxia. Epo mRNA was upregulated in all the cell lines, and in G361, MCF-7 and C32TG it was significantly upregulated under anoxia for 6 h ($P<0.01$, $P<0.05$) (Fig. 1B). The fold induction of Epo mRNA in G361, MCF-7 and C32TG was approximately 9.5-, 4.8- and 1.8-fold, respectively, which corresponds to the band intensity for HIF-1α

under anoxia [24]. The Epo mRNA in hepatocytes was not detectable under normoxia, but under anoxia it was induced significantly ($P<0.01$), while, in NHDF, it was as low as 0.04 ± 0.00 (Fig. 1B) under normoxia without anoxia-inducibility. The value of NHDF was 48.8-fold less than that of G361, which expressed the least Epo mRNA under normoxia indicating that malignant cells express over 50-fold the Epo mRNA of normal cells.

The content of Epo protein in each culture medium for 24 h under normoxia ranged from 0.04 ± 0.03 in MCF-7 to 13.4 ± 0.01 mU/mg protein in SCH. It was higher under anoxia in all cell lines; in nine of them it was significantly higher ($P<0.05$, $P<0.01$, $P<0.001$; Fig. 2A). These cancer cell lines express HIF-1α under anoxia, except for SCH [24]. Normal serum Epo levels are 5 to 25 mU/ml [1], or 0.07 to 0.35 mU/mg of serum protein. The majority of the cell lines secreted less than the upper normal level (Fig. 2A), however, G361, C32TG, SBC3, AZ521 and SCH under normoxia and anoxia, and K562, P22 and T98G under anoxia secreted more than normal (Fig. 2A). Anoxia raised the secretion in hepatocytes and NHDF, but not higher than normal (Fig. 2A). Under normoxia, the order of expression of Epo mRNA did not always correspond to that of Epo protein. G361 and P39 expressed low Epo mRNA, but they secreted more Epo, especially G361. However, the eight cell lines that secreted above the normal range generally had high Epo mRNA levels (Fig. 1A, 2A). Consequently, the majority of the malignant cell lines appear to secrete Epo protein, as indicated by the levels of expression of Epo mRNA.

3.2 Expression of EpoR in malignant cell lines.

The EpoR mRNA levels ranged from 92.02 ± 8.47 in WiDr to $49{,}829.69 \pm 3{,}823.76$ in UT-7; these were 8×10^2 - 3×10^6 higher than those of Epo mRNA (Fig. 1A). The content of EpoR mRNA in hepatocytes was 0.45 ± 0.07 and in NHDF 1.42 ± 0.23, which was 547- and 65-fold less than that of HepG2 and WiDr, respectively (Fig. 1C). The expression of EpoR mRNA in C32TG, G361, HepG2 and A172 rose significantly under anoxia ($P<0.05$, $P<0.01$, Fig. 1C), and the upregulation of EpoR in C32TG, G361, HepG2 and A172 was approximately 4.0-, 4.0-, 2.8- and 3.4-fold, respectively. Although, anoxia upregulated the EpoR mRNA in hepatocytes ($P<0.01$) the level of expression was 195-fold less than that of HepG2 (Fig. 1C). These data indicated that EpoR mRNA is expressed much more than Epo mRNA in malignant cell lines and that anoxia upregulates it frequently in tumors of ectodermal origin, except for endodermal hepatoma. Moreover, EpoR mRNA is expressed 50- to 500-fold more in malignant than in normal cells.

Western blot analysis of the membrane fraction of 24 cell lines under normoxia (Fig. 2B upper panel), and 4 cell lines in which EpoR mRNA was elevated under anoxia (Fig. 2B lower panel) showed a band with various intensities for EpoR under normoxia as the positive control of UT-7 [25] (Fig. 2B upper panel). The band intensity did not always parallel the low to high expression order of EpoR mRNA of the cell lines (Fig. 2B upper panel). Stronger bands were seen under anoxia for 24 h than under normoxia in G361 and C32TG, but similar band intensity was detectable in HepG2 and A172, the bands of which were stronger than those of the other two cell lines under normoxia (Fig. 2B lower panel).

3.3 rhEpo-induced growth of malignant cell lines.

The proliferation of seven cell lines treated with various doses of rhEpo once a day in medium changed every day for four days was determined by the uptake of IdU. Dose-response curves of each cell line were plotted (Fig. 3A). All cell lines responded significantly to rhEpo with large or small

increases, but there were various responses to graded doses of rhEpo (Fig. 3A); five of them showed dose-dependency to rhEpo; the SCH cell line responded maximally to less than 2.5 U/ml of rhEpo ($P<0.01$), and T98G and P39 responded slightly without dose-dependency ($P<0.05$). Up to 25 U/ml, HepG2, G361 and DLD1 showed a monophasic growth pattern while that of SCH and PC-3 was biphasic (Fig. 3A). The fold-increase in each cell line was plotted, and the uptake values were compared at various concentrations with a control culture which is plotted as 1.0 (Fig. 3B). Up to 25 U/ml, growth was stimulated approximately 1.2 - 1.7-fold of the control rate (Fig. 3B).

3.4 EMP9-reduced growth of malignant cell lines.

Seven malignant cell lines were treated with various doses of EMP9 4 times at 1-h intervals, and their growth was measured at 24 h and the dose-response curves of each cell line were plotted (Fig. 3C). Six cell lines responded significantly up to 1.0 mg/ml, and SCH, to 5.0 mg/ml of EMP9 with a reduction in growth; the growth of six of them was reduced dose-dependently; the DLD1 showed reduced growth dose-dependently with less than 0.25 mg/ml of EMP9 ($P<0.001$) (Fig. 3C), but the HepG2 responded differently. The fold-decrease in each cell line was compared with that of a control culture, plotted as 1.0. Exposure to EMP9 reduced growth by 0.3 - 0.8-fold of the control in six cell lines receiving 1.0 - 2.0 mg/ml, and by 0.75-fold in SCH, receiving 5.0 mg/ml (Fig. 3D).

3.5 Epo-signaling in the cell lines.

Because of the data of rhEpo-induced and EMP9-reduced growth shown in Figure 3C, we selected 5 U/ml of rhEpo, and 0.25 and 0.5 mg/ml of EMP9, respectively, to determine their effect on Epo-signaling. The band intensity was expressed as relative to the strongest expression in the band for P-Tyr and STAT5 of each cell line (Fig. 3E). The expression of STAT5 was comparable in six of the seven cell lines; PC-3 showed slightly weaker expression than the others (Fig. 3E). Then, we compared the expression levels of P-Tyr among the seven cell lines. Surprisingly, the cell lines cultured without stimulator expressed activated tyrosine phosphorylation in STAT5: the high to low activation order was SCH, HepG2, DLD1, G361, P39, T98G and PC-3 (Fig. 3E). rhEpo induced the strongest activation of tyrosine phosphorylation in STAT5 protein of the six cell lines except for SCH. EMP9 suppressed the activation of P39, PC-3, T98G, G361, HepG2, DLD1 and SCH in strong to weak order (Fig. 3E). Stimulation by rhEpo after EMP9 exposure reversed the activation levels in five cell lines, T98G, HepG2, SCH, PC-3 and P39, but in DLD1 and G361, it did not reverse the levels, which were comparable with or half the value of the controls, respectively (Fig. 3E). Taken together, Epo-signaling triggered by rhEpo, suppressed by EMP9 and operated through autocrine stimulation by Epo was demonstrated to enhance malignant cell growth.

4 Discussion

The present quantitative data of the expression levels of transcripts for Epo and EpoR and of Epo protein with EpoR protein in each malignant cell line provide precise evidence of how the growth of various malignant tumors is promoted differently through their own Epo-EpoR signaling. Moreover, the evidence that a majority of cell lines expressed their own constitutive activation of tyrosine phosphorylation of STAT5, enhanced by rhEpo and suppressed by EMP9, indicates the substantial role of Epo signal in tumor growth.

Epo mRNA is expressed in various amounts in 24 malignant cell lines under normoxia. The difference in Epo mRNA expression appears to

reflect the cell line's original organ before transformation, i.e. its location in the body. Previously, we found that eight of the nine cell lines from tumors of ectodermal origin express HIF-1α under anoxia and hypoxia, but all cell lines of gastrointestinal tumors except for the esophagus do not express it [24]. The cell lines without HIF-1α expressed higher Epo mRNA and/or secreted much higher Epo protein than did those with HIF-1α, except for KYSE170 and KYSE220 (Fig. 1A, 2A). It is reasonable to consider that the tumors derived from tissues located superficially or in the upper digestive tract are usually exposed to air containing abundant oxygen through the skin or mucous membrane of the esophagus so they do not need as much Epo to survive and proliferate as do those located deeply in the body. However, when the supply decreases or the demand increases, they need much more Epo than do the tumors secreting more Epo under normoxia. The evidence that T47D does not express HIF-1α under anoxia [24] and secretes more Epo than does MCF-7, which expresses HIF1-α (Fig. 1A), must be taken into consideration.

The growth activity of the seven selected cell lines cultured for 96 h with or without rhEpo was highest in SCH, followed in order by PC-3, DLD1, G361, HepG2, P39 and T98G (Fig. 3A). This order corresponds neither to the expression order of the EpoR mRNA (Fig. 1A) nor the band intensity of the EpoR protein (Fig. 2B), suggesting that the promotion of growth does not depend solely on the level of EpoR mRNA seen in erythroblasts. In the present study, all selected cell lines exhibited their own levels of constitutive activation of tyrosine phosphorylation, the order being SCH, HepG2, DLD1, G361, P39, T98G and PC-3 (Fig. 3E); the activation levels in HepG2, DLD1 and G361 were comparable, thus far, and corresponded well to the growth activity order except for PC-3, in which rhEpo triggered it the highest, by up to 500-fold. Feldman et al. [26] demonstrated that in PC-3, STAT5a was almost undetectable, but STAT5b was seen, concomitant with the phosphorylation of STAT5b. In the present study, STAT5 was detectable in PC-3 as a relatively strong band, but phosphotyrosine appeared extremely weak. The difference from Feldman's data and the weak expression of tyrosine phosphorylation remain to be analyzed further. Consequently, exogenous rhEpo promotes the growth of the majority of malignant cells depending on the level of expression of endogenous Epo-signal which may show differences in growth rate.

EMP9 inhibited the Epo signal in all selected cell lines by suppressing activation of tyrosine phosphorylation; this was reversed by rhEpo. In three of them it was higher and in the other four it was lower than the constitutive activation level (Fig. 3E). These reversions by rhEpo may or may not reflect growth promotion. Further clarification is required. However, significant reduction in growth due to EMP9 exposure was seen in DLD1 at 0.25 mg/ml ($P<0.001$), and in HepG2 ($P<0.001$), P39 ($P<0.001$), PC-3 ($P<0.05$), T98G ($P<0.05$) and G361 ($P<0.01$) at 0.5 mg/ml (Fig. 3C). The high to low growth reduction order appears to be DLD1, HepG2, P39, PC-3, T98G, G361 and SCH (Fig. 3D); this order did not correlate with the order of the level of suppression of the constitutive activation of tyrosine phosphorylation, but did correspond to the low to high Epo secretion order except for T98G and PC-3, in which Epo content were comparable (Fig. 2A). Thus EMP9 appears to reduce the growth of malignant cell lines depending inversely on the secretion levels of Epo protein.

Some reports have noted results opposite to ours; rhEpo did not promote the growth of cell lines under various concentration of rhEpo [27,28]. The differences may be due to our culture conditions;

changing the rhEpo-containing medium every 24 hr and detecting the P-Tyr in STAT5 protein not in the lysates [28].

In conclusion, various cancer cells in general appear not only to express Epo and EpoR in both transcript and protein levels individually but also to acquire their own constitutive Epo-signaling in operation that regulates their growth differently.

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Figure legends

Fig. 1 Expression of Epo and EpoR mRNA in cell lines. A. Individual expression levels of Epo and EpoR mRNA compared with those of 18S rRNA mRNA displayed as mean ± SEM of values; the number in each bar indicates the fold-expression to that of Epo mRNA; B. Significant upregulation of

Epo mRNA under anoxia (*, P<0.05, **, P<0.01); C. Significant upregulation of EpoR mRNA under anoxia(*, P<0.05, **, P<0.01).

Fig. 2 Content of Epo protein and expression of EpoR protein. A. Epo protein levels in the culture medium of malignant cell lines and normal cells under normoxia and anoxia for 24 h. The two horizontal dotted lines are the levels of normal Epo in serum protein. *, **, ***, Significant differences between normoxia and anoxia by Student's t-test. a, Significant differences in Epo values between PC-3 and G361 under normoxia. *, P<0.05, **, P<0.01, ***, P<0.001, a, P<0.05; B. Western blot analyses of EpoR protein of 24 cell lines cultured under normoxia. The bands for EpoR protein are detectable under normoxia as the positive control of UT-7 in the upper panel; stronger bands are seen under anoxia (a) than under normoxia (n) in two cell lines cultured for 24 h in the lower panel.

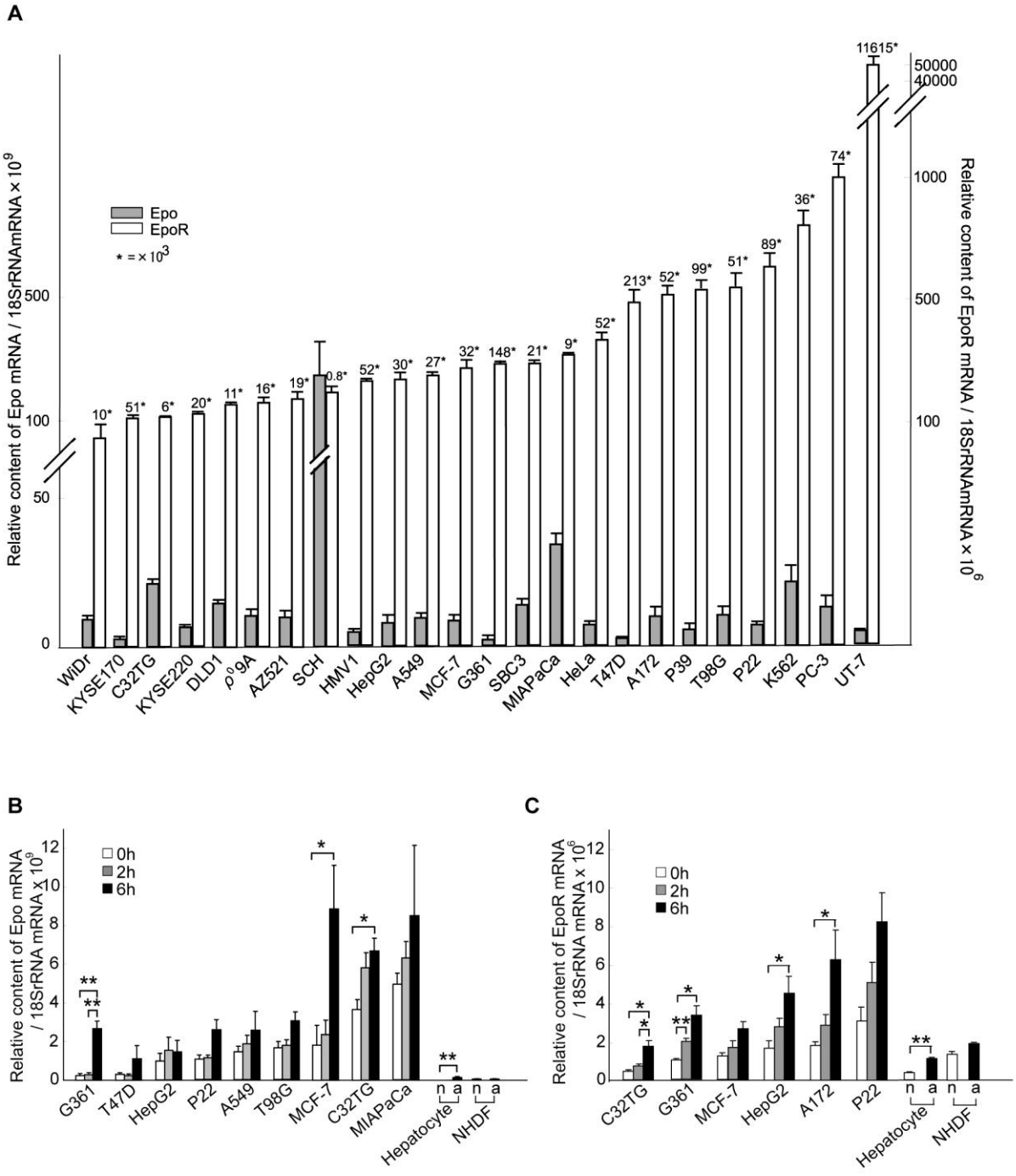
Fig. 3 Effects of rhEpo and EMP9 on growth. A. Cell lines were exposed to rhEpo (2.5-25 U/ml) for 4 d; B. The fold-increase in each cell line was plotted as 1.0 for growth of each cell line without rhEpo; C. Cell lines were exposed to EMP9 (0.1-5 mg/ml) four times at 1 h intervals for 24 h; D. The fold-decrease in each cell line was plotted as 1.0 for growth of each cell lines without EMP9. Data are expressed as mean ± SEM of triplicate determinations with background subtracted. The letter on the dot means significant differences from the value at 0 U/ml (a), 2.5 U/ml (b), 5.0 U/ml (c) and 10.0 U/ml (d). *, **, ***, Significant difference from the value for each letter by Student's t-test, *, P<0.05, **, P<0.01, ***P<0.001; E. Tyrosine phosphorylation of STAT5 in cell lines exposed to rhEpo, or EMP9 or EMP9 followed by rhEpo. Immunoprecipitation and western blots were done as described under Materials and Methods. Numbers under each band indicate relative amount of intensity. M, molecular weight markers.

Table. 1 Origin and characteristics of cell lines

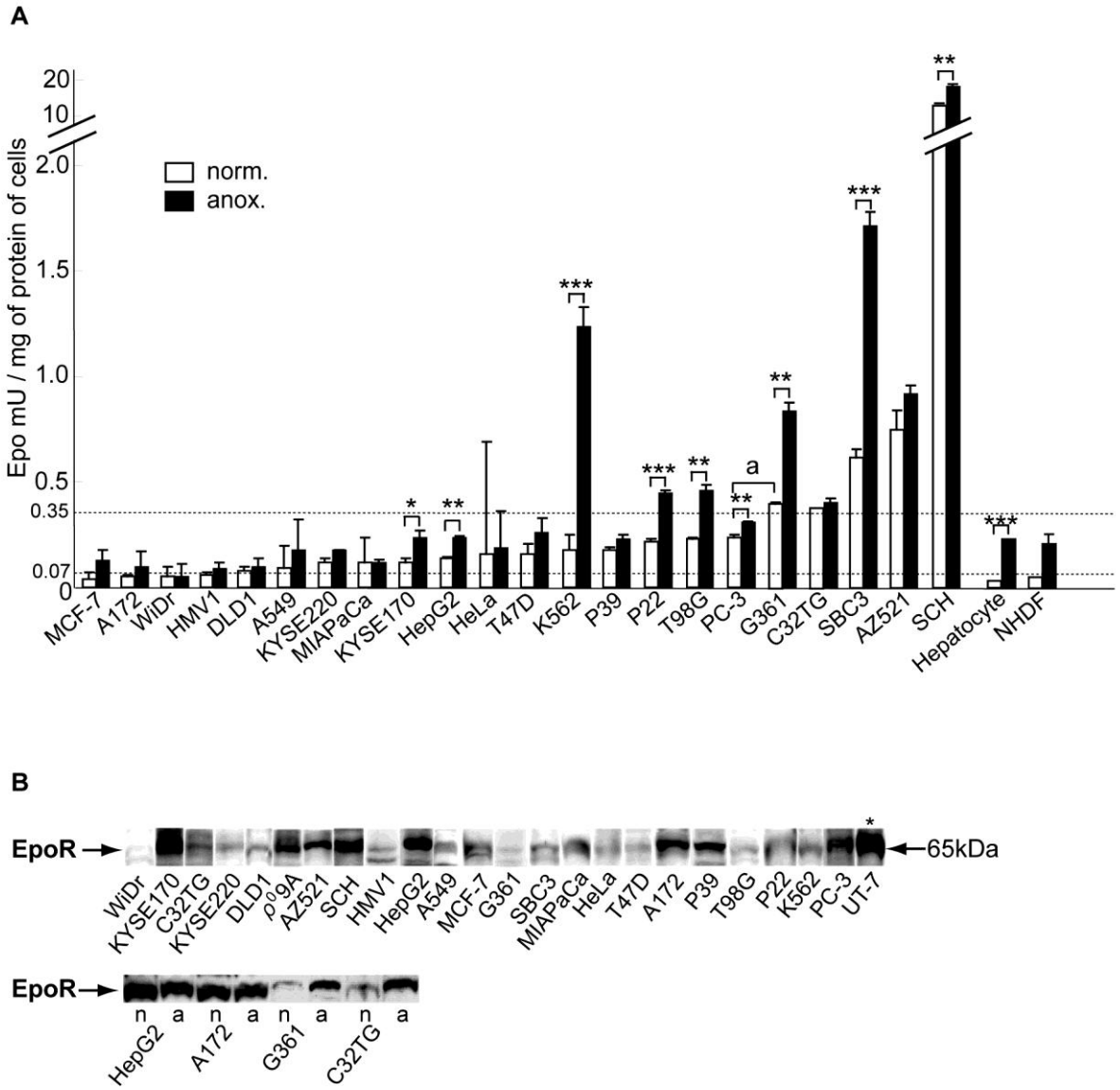
Cell name	Origin and characteristics	Parental developmental origin
T98G	Glioblastoma, $p53^-$	EC
A172	Glioblastoma, $p53^+$	EC
G361	Melanoma, radiation resistant	EC
P39	Melanoma, radiation sensitive	EC
HMV1	Melanoma, vaginal origin	EC
C32TG	Melanoma, amelanotic	EC
P22	Melanoma	EC
KYSE170	Esophageal SCC, CDDP resistant	EN
KYSE220	Esophageal SCC, CDDP sensitive	EN
AZ521	Gastric cancer, epitheliar type	EN
SCH	Stomach, choriocarcinoma	EN
DLD1	Colon adenocarcinoma	EN
Widr	Colon adenocarcinoma	EN
A549	Lung adenocarcinoma	EN
SBC3	Lung, small cell cancer	EN
T47D	Mammary cancer	EC
MCF-7	Breast carcinoma	EC
HepG2	Hepatoma	EN
MIAPaCa	Pancreatic cancer	EN
HeLa	Cervical epidermoid cancer	MS
$\rho^0$9A	Mutant of HeLa, $mtDNA^-$	MS
PC-3	Prostate adenocarcinoma	MS
K562	Leukemia	MS
UT-7	Leukemia	MS
Hepatocyte	Human primary hepatocyte cells (ACBRI) (ABI-3716)	EN
NHDF	Normal human dermal fibroblasts (Canbrex) (CC2511)	MS

EC, ectoderm; MS, mesoderm; EN, endoderm

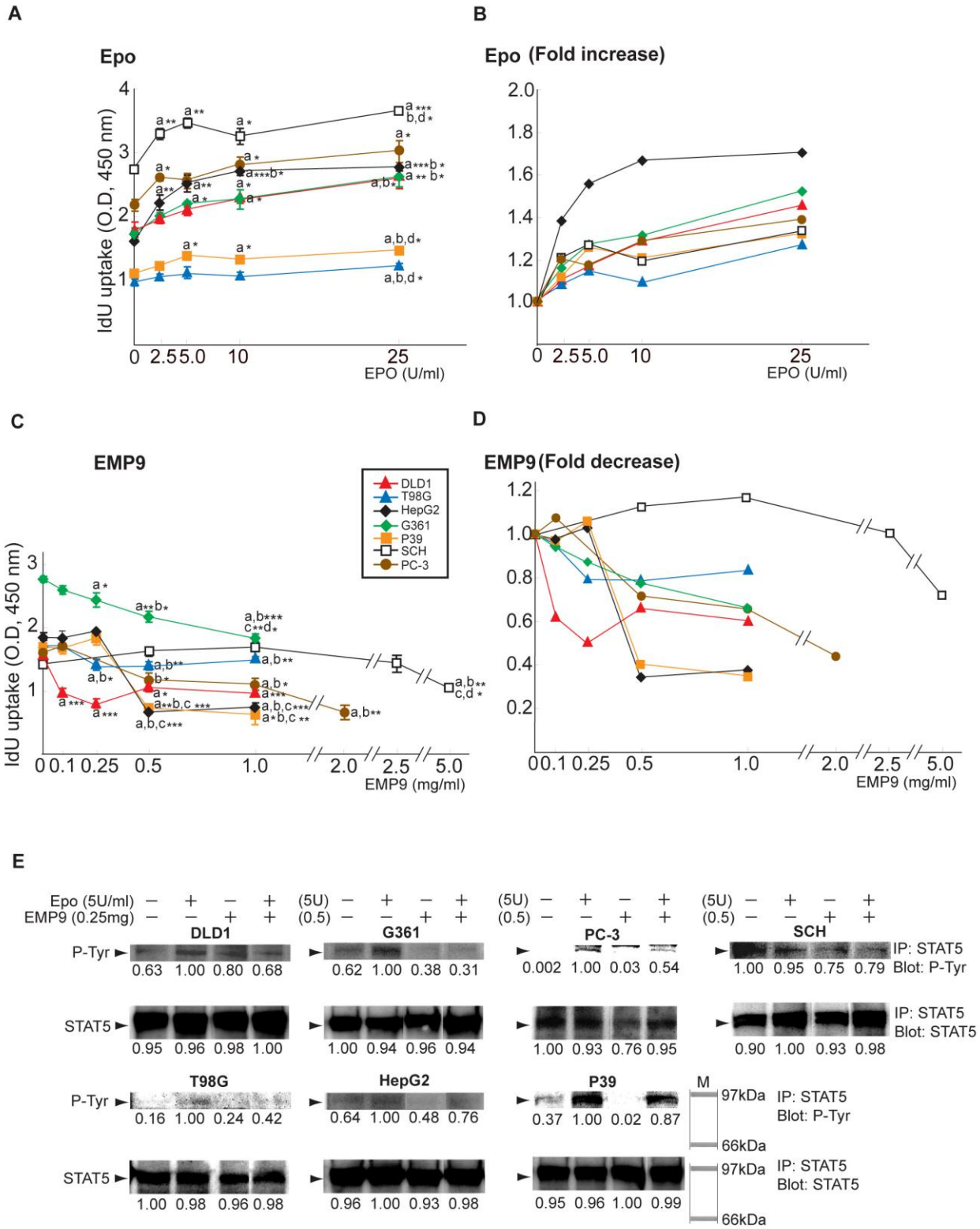
WSEAS
Fig. 1


A
Relative content of Epo mRNA / 18SrRNAmRNA x 10^9
Relative content of EpoR mRNA / 18SrRNAmRNA x 10^6
Epo
EpoR
* = x 10^3
WiDr
KYSE170
C32TG
KYSE220
DLD1
ρ0 9A
AZ521
SCH
HMV1
HepG2
A549
MCF-7
G361
SBC3
MIAPaCa
HeLa
T47D
A172
P39
T98G
P22
K562
PC-3
UT-7
B
Relative content of Epo mRNA / 18SrRNA mRNA x 10^9
0h
2h
6h
G361
T47D
HepG2
P22
A549
T98G
MCF-7
C32TG
MIAPaCa
Hepatocyte
NHDF
C
Relative content of EpoR mRNA / 18SrRNA mRNA x 10^6
C32TG
G361
MCF-7
HepG2
A172
P22
Hepatocyte
NHDF

WSEAS
Fig. 2


A
Epo mU / mg of protein of cells
20
10
2.0
1.5
1.0
0.5
0.35
0.07
0
norm.
anox.
MCF-7
A172
WiDr
HMV1
DLD1
A549
KYSE220
MIAPaCa
KYSE170
HepG2
HeLa
T47D
K562
P39
P22
T98G
PC-3
G361
C32TG
SBC3
AZ521
SCH
Hepatocyte
NHDF
B
EpoR
65kDa
WiDr
KYSE170
C32TG
KYSE220
DLD1
ρ0gA
AZ521
SCH
HMV1
HepG2
A549
MCF-7
G361
SBC3
MIAPaCa
HeLa
T47D
A172
P39
T98G
P22
K562
PC-3
UT-7
EpoR
n a n a n a n a
HepG2
A172
G361
C32TG

WSEAS
Fig. 3


A
Epo
IdU uptake (O.D, 450 nm)
EPO (U/ml)
B
Epo (Fold increase)
EPO (U/ml)
C
EMP9
DLD1
T98G
HepG2
G361
P39
SCH
PC-3
IdU uptake (O.D, 450 nm)
EMP9 (mg/ml)
D
EMP9 (Fold decrease)
EMP9 (mg/ml)
E
Epo (5U/ml)
EMP9 (0.25mg)
(5U)
(0.5)
DLD1
G361
PC-3
SCH
P-Tyr
0.63 1.00 0.80 0.68
0.62 1.00 0.38 0.31
0.002 1.00 0.03 0.54
1.00 0.95 0.75 0.79
IP: STAT5
Blot: P-Tyr
STAT5
0.95 0.96 0.98 1.00
1.00 0.94 0.96 0.94
1.00 0.93 0.76 0.95
0.90 1.00 0.93 0.98
IP: STAT5
Blot: STAT5
T98G
HepG2
P39
M
0.16 1.00 0.24 0.42
0.64 1.00 0.48 0.76
0.37 1.00 0.02 0.87
97kDa
66kDa
IP: STAT5
Blot: P-Tyr
1.00 0.98 0.96 0.98
0.96 1.00 0.93 0.98
0.95 0.96 1.00 0.99
97kDa
66kDa
IP: STAT5
Blot: STAT5