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Effects of Recombinant Human Erythropoietin on Clonogenic Growth of Primary Human Tumour Specimens *in vitro*

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BESIDES RENAL anaemia, anaemia of malignancy is a potential target for the clinical use of recombinant human erythropoietin (rhE). However, concerns of potential tumour stimulating effects arise from *in vitro* data. In particular, renal cancer cell lines have been reported to secrete erythropoietin or erythropoietin-like factors into their conditioned medium [1–3]. We report here the effects of human recombinant erythropoietin on clonal growth of freshly explanted cells from a variety of cancer types.

Tumour specimens from 151 patients were obtained by sterile standard techniques as part of routine surgical procedures. Single cell suspensions were prepared by mechanical dissociation. rhE (Erypo®) was kindly provided by Cilag Inc. (Sulzbach, FRG) at a specific activity of 1.2×10^5 U/mg. Final concentrations of 0.4, 4.0, 40.0 and 400.0 U/ml were used and were prepared in phosphate-buffered saline containing 0.1% bovine serum albumin (PBS/BSA). Human recombinant granulocyte–macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), and interleukin 3 (IL-3) were gifts from Behringwerke AG (Marburg, FRG) and had a specific activity of 5×10^7 U/mg. IL-1α and IL-1β were purchased from Amersham (Braunschweig, FRG) and were provided at specific activities of $\geq 5 \times 10^8$ U/mg. IL-6 was a gift from K. Welte, Department of Pediatrics, Medical School of Hannover (FRG). A capillary soft-agar cloning system was used as described [4]. 53 specimens showed adequate growth in controls. The major subgroups of tumours studied were renal cell cancer and colorectal cancer.

Table 1. Effects of recombinant human erythropoietin on in vitro colony formation of primary human tumour specimens

	0.4 U/ml	4 U/ml	40 U/ml	400 U/ml
Stimulation*				
Renal	0/18	0/23	0/23	0/23
Colorectal	0/5	0/13	0/13	0/13
Others	0/10	2/17	2/17	0/17
Inhibition†				
Renal	0/18	1/23	1/23	2/23
Colorectal	0/5	0/13	0/13	2/13
Others	0/10	0/17	0/17	1/17

* No. specimens with stimulation/no. specimens with growth in control capillaries. An increase in colony formation to $\geq 1.5 \times$ control was considered a significant stimulation.
† No. specimens with inhibition/no. specimens with growth in control capillaries. A decrease of colony formation to $\leq 0.5 \times$ control was considered a significant inhibition.

Without erythropoeitin, the median number of colonies formed per capillary was 10.0 (range 3.0–274.0). The frequency of growth modulating effects by rhE in all evaluable specimens is shown in Table 1. Two of 53 evaluable specimens (3.8%) (soft tissue sarcomas) showed a significant stimulation of colony formation by rhE at final concentrations of 4 and 40 U/ml. However, no clear concentration response was observed in these specimens. None of 63 specimens with insufficient growth in control capillaries was converted to evaluable growth by rhE.

Placebo controls containing the solvent solution of the commercial rhE preparation were studied in 14 evaluable tumour specimens. No significant growth modulation was observed (data not shown). There was no evidence for synergistic or additive effects when rhE (200 U/ml) was combined with IL-1α, IL-1β, IL-6 (all at 10 ng/ml) or IL-3, G-CSF, GM-CSF (all at 100 ng/ml) (data not shown).

Our data provide evidence that erythropoietin is not a growth modulator for human cancer cells. In concordance with earlier findings, growth of renal cancer cells was not stimulated by GM-CSF, G-CSF or IL-3 *in vitro* [5]. Also, we found no evidence for an additive or a synergistic action of rhE and IL-1α, IL-1β, IL-3, IL-6, GM-CSF or G-CSF on colony formation of human tumour cells *in vitro*. We conclude that rhE may be safely administered to cancer patients without concern of tumour stimulation.

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