Cancer Immunol Immunother (1993) 37: 150–156

Cancer Immunology Immunotherapy

Cytosine arabinoside increases the binding of ^{125}I-labelled epidermal growth factor and ^{125}I-transferrin and enhances the in vitro targeting of human tumour cells with anti-(growth factor receptor) mAb

Michele Caraglia[1], Pierosandro Tagliaferri[1], Pierpaolo Correale[1], Geppino Genua[1], Antonio Pinto[2], Silvana Del Vecchio[3], Giuseppe Esposito[3], Angelo Raffaele Bianco[1]

[1] Cattedra di Oncologia Medica, Facoltà di Medicina, Università degli Studi "Federico II" di Napoli, Italy
[2] Centro di Riferimento Oncologico, Aviano (PN), Italy
[3] Istituto Nazionale dei Tumori, Fondazione "G. Pascale", Napoli, Italy

Received: 21 October 1992/Accepted: 2 February 1993

Abstract. We report that cytosine arabinoside (Ara-C), a cytosine analogue that at low doses causes phenotypical changes on human leukemia cells in vitro and in vivo, induces growth inhibition of oropharyngeal cancer KB and lung adenocarcinoma A549 cell lines. An increase in the number of epidermal growth factor and transferrin receptors (EGFR, TrfR) is induced by Ara-C on these cells. Maximal EGFR up-regulation occurs 96 h after the beginning of Ara-C exposure while maximal TrfR up-regulation is detected 24 h later. These effects occur without changes in the affinity of EGFR and TrfR for their ligands. Two classes of EGF-binding sites with a K_d of 0.055 nM and 2.3 nM respectively, and one class of transferrin-binding sites with a K_d of about 4 nM are detected on both untreated and Ara-C-treated KB cells. [^{3}H]Thymidine uptake is clearly stimulated on KB cells by nanomolar concentrations of EGF and transferrin, whereas in Ara-C-treated cells [^{3}H]thymidine uptake is not increased by EGF and transferrin under conditions where maximal EGFR and TrfR up-regulation occurs. The enhanced EGF and transferrin binding is paralleled by a twofold increase of in vitro targeting of Ara-C-treated KB and A549 cells with anti-EGFR 108.1 mAb and anti-TrfR OKT9 mAb. We propose that Ara-C could provide a new approach for the improvement of the therapeutic index of anti-EGFR and anti-TrfR immunoconjugates.

Key words: Ara-C – Tumour cells – EGFR – Transferrin receptor – Immunotargeting

Introduction

Monoclonal antibodies (mAb) that have been generated against tumour-associated antigens (TAA) are considered an important tool for diagnosis and therapy of human tumours. In fact they could allow the selective delivery of drugs and radionuclides to the tumour sites [10, 22].

Cellular receptors for epidermal growth factor (EGFR) and transferrin (TrfR) are presently considered as TAA, since they are highly expressed by several human cancers and barely detectable in most normal tissues [13, 16, 21, 24, 40, 42].

Moreover, these receptors are important regulators of tumour cell growth. EGF is a potent mitogen for epithelial tumour cells in vitro and in vivo and its receptor is considered the cellular effector of transforming growth factor α (TGFα), an autocrine growth factor produced by tumour cells [5, 7, 35]. On the other hand, the expression of TrfR is associated with the proliferative status of tumour cells. Many studies on cell lines grown in serum-free media have demonstrated that transferrin or other Fe^{2+} sources are required for cellular proliferation [27, 39]. Furthermore, the structure and function of EGFR and TrfR have been studied in detail while much less is known for most of the other TAA [34, 41]. Therefore, EGFR and TrfR appear an excellent tool for new experimental approaches for the enhancement of selective cancer cell targeting with drug- or radionuclide-conjugated mAb [24, 41]. Recently, genetically engineered fusion proteins have been generated that could allow the selective delivery of bacterial toxins to tumour cells through binding to growth factor receptors expressed at the cell surface. EGFR- and TrfR-targeted fusion proteins are presently being investigated as anticancer agents by several groups [28].

Theoretical and experimental findings have recently suggested that antigen density on tumour cells could play a critical role in the regulation of mAb uptake by cancer cells. The number of available mAb binding sites at the tumour cell surface appears a more critical determinant than mAb affinity itself for the efficiency of the targeting process. These observations suggest an advantage for the

This work has been supported by the Italian Association for Cancer Research (A.I.R.C.) and by the National Council of Research (C.N.R.) of Italy, contract 92.02274.PF39

Correspondence to: M. Caraglia, Cattedra di Oncologia Medica, Facoltà di Medicina, Università degli Studi "Federico II" di Napoli, Via S. Pansini 5, 80131, Napoli, Italy

use of mAb raised against TAA highly expressed at the tumour cell surface [14, 37, 38]. The increase of TAA density on cancer cells by the use of exogenous agents could be, in this case, of major clinical benefit. For these purposes, interferons (IFN) have been reported to be powerful modulators of TAA expression at the tumour cell surface and enhance in vivo targeting of cancer cells with radionuclide-conjugated mAb [15, 31]. On the other hand, retinoic acid has been reported to induce EGFR up-regulation on human tumour cells [19]. We have recently found that IFNα exerted similar effects on human epidermoidal cancer cells and we have speculated that the increased expression of growth factor receptors could be part of a homeostatic cellular response to the strong antiproliferative effect of such an agent [4]. We have thus hypothesized that other agents that induce a strong cytostatic effect could also enhance the surface expression of cellular receptors for EGF and modify the expression of other peptide growth factor receptors. Cytosine arabinoside (Ara-C) is a cytosine analogue which, at low doses, inhibits proliferation of human leukaemic and solid tumour cells without affecting cell viability [12] (and see below). Moreover, Ara-C induces phenotypic changes on human myeloid leukaemic cells [30]. The aim of this work was the study of the effects of Ara-C on binding of EGF and transferrin by human oropharyngeal KB and lung A549 cancer cells and on specific targeting of these cells in vitro with anti-EGFR and anti-TrfR mAb. We selected cells derived from human head and neck and lung cancers because these cells commonly present high expression of EGFR and are considered as suitable targets for immunotherapy strategies based on anti-EGFR mAb [1, 17]. An important point was to define whether Ara-C-induced modulation of receptor expression could be associated with changes in the sensitivity of tumour cells to EGF and transferrin. Definition of modulating effects exerted by Ara-C on the expression of growth factor receptors could allow the study of mechanisms of tumour cell growth regulation and, at same time, provide the basis for new immunotargeting approaches.

Materials and methods

Cell culture and cell proliferation assay. The human oropharyngeal epidermoid carcinoma KB cell line, provided by Prof. S. Bonatti (Facoltà di Medicina, Università di Napoli, Italy), was grown in Dulbecco's modified Eagle medium (DMEM) supplemented with heat-inactivated 10% fetal bovine serum FBS, 20 mM HEPES, 1% penicillin/streptomycin, 1% L-glutamine and 1% sodium pyruvate. The human lung adenocarcinoma A549 cell line, purchased from American Type Culture Collection, Rockville Md., was maintained in DMEM supplemented with 10% FBS, 20 mM HEPES, 1% penicillin/streptomycin and 1% L-glutamine. The cells were grown in a humidified atmosphere of 95% air/5% CO_2 at 37° C. For cell growth experiments, A549 and KB cells were seeded at 2×10^5 and 3×10^5 cells per well, respectively, in 6-well culture plates (Nunc). The medium was removed 24 h later and fresh medium containing Ara-C was added every 24 h thereafter. At selected times cell growth assessment was performed by haemocytometric cell counting and a trypan blue viability assay, following gentle trypsinization.

Radiolabelled preparations. Transferrin was labelled with ^{125}I to a specific activity of approximately 1 μCi/μg using the Iodogen method [32]. The radiolabeled protein was purified from unbound iodide by Sephadex G-25 chromatography (Pharmacia).

^{125}I-EGF and ^{125}I-labelled sheep anti-(mouse Ig) IgG were purchased from Amersham.

^{125}I-EGF and ^{125}I-transferrin radiobinding experiments. KB and A549 cells were seeded in 24-well plates at 2×10^4 and 10^4 cells/well respectively. The treatment with Ara-C was performed as described above. After overnight incubation in serum-free medium (i.e. DMEM with non-essential amino acids and vitamins added) in the presence or absence of Ara-C, the cells were washed twice with ice-cold phosphate-buffered saline (PBS) and, after the addition of 1 mg/ml bovine serum albumin (BSA), they were incubated for 4 h at 4° C with 200 μl/well binding buffer (DMEM, 25 mM HEPES, 1 mg/ml BSA) containing increasing concentrations of ^{125}I-EGF (164 μCi/μg) or ^{125}I-transferrin (200 μCi/μg), as previously described [8]. After incubation, cells were washed four times with PBS/BSA and lysed in 0.5 ml/well 20 mM HEPES, 1% Triton X-100, 10% glycerol. Cell-associated radioactivity was counted in a Beckman gamma counter.

The non-specific binding, determined by the addition of a 100-fold excess of unlabelled EGF and transferrin and then subtracted for each point, never exceeded 5%. The EGFR and TrfR binding affinity and the receptor number were determined by Scatchard analysis of EGF and transferrin binding data [33], using the EBDA/LIGAND program for fitting multiple-binding-site data [26].

EGFR and TrfR live-cell radioimmunoassay. Live-cell RIA experiments were performed with the anti-EGFR 108.1 (provided by Dr. J. Schlessinger) and the anti-TrfR OKT9 (purchased by Ortho, Raritan, N.J.) prepared as previously described [2, 36].

KB and A549 cells were harvested from subconfluent cultures and seeded in 96-well microtiter plates at 5×10^3 and 2×10^3 cells/well respectively. The treatment with Ara-C was performed as described above. After overnight incubation in serum-free medium (DMEM with non-essential amino acids and vitamins added) in the presence or absence of Ara-C, the growth medium was removed and 100 μl 5% BSA (w/v) in DMEM and 0.08% (w/v) sodium azide were added to each well. After 60 min of incubation at 37° C, the medium was removed and cells were washed with DMEM containing 5% BSA and 0.08% sodium azide. Samples containing 50 μl appropriately diluted mAb 108.1 or mAb OKT9 were then added to each well. After incubation for 3 h at 4° C, mAb 108.1 and mAb OKT9 were removed and the cells were washed twice with 5% PBS/BSA (w/v). Then 75 000 cpm in 50 μl ^{125}I-labelled sheep anti-(mouse Ig) IgG were added to each well for 60 min at 37° C. Following incubation, cells were washed three times with PBS/BSA 5% (w/v) and 50 μl 2 M NaOH was added to each well and adsorbed with a cotton swab and radioactivity was counted in a Beckman gamma counter. The background from the well that received only buffered DMEM was approximately 100–250 cpm, and was subtracted from that of the wells exposed to mAb 108.1 and the mAb OKT9 as previously described [15].

[^{3}H]Thymidine uptake. KB cells were seeded in 24-well plates (Nunc) at 2×10^4 cells/well. Cells were exposed to Ara-C as described above. After overnight incubation at 37° C with escalating concentrations of EGF or transferrin, 5 μCi [*methyl*-^{3}H]thymidine (Amersham, 5 Ci/mmol) was added. After 6 h incubation at 37° C, KB cells were washed twice with PBS and lysed in 0.5 ml/well 20 mM HEPES, 1% Triton X-100, 10% glycerol. Cell-associated radioactivity was counted in a Beckman beta counter (LS 1801), as previously described [27].

Results

Ara-C induces antiproliferative effects on KB and A549 cells

In cell-proliferation assays, 50% growth inhibition (IC_{50}) was achieved on KB and A549 cells after 96 h exposure to 1000 nM and 10 nM Ara-C respectively (Fig. 1 A). The

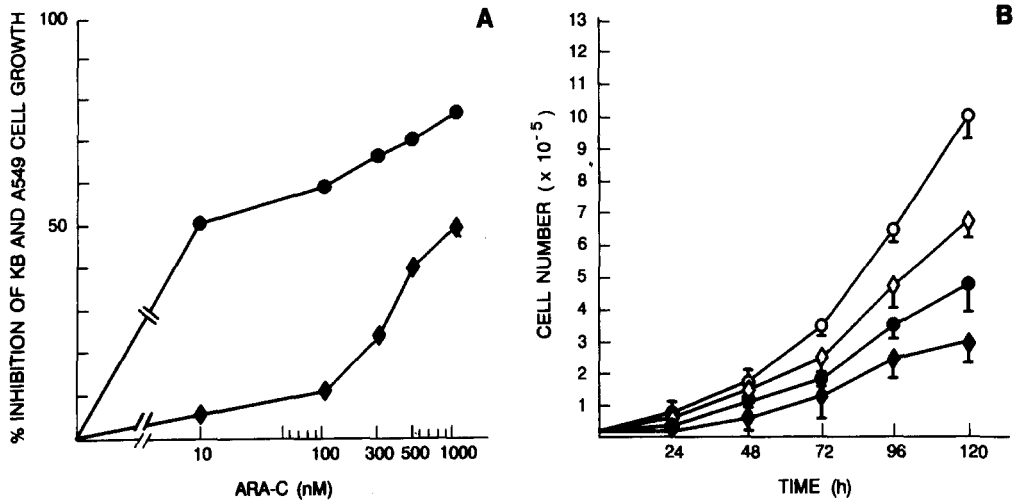

Fig. 1 A, B. Effects of cytosine arabinoside (Ara-C) on the growth of KB and A549 cancer cells. **A** Effects of different concentrations of Ara-C on the growth of KB (◆) and A-549 (●) cells after 96 h exposure to the drug. Growth inhibition is expressed as a percentage of untreated controls. **B** Cell growth of untreated KB cells (◇) and KB cells treated with 1000 nM Ara-C (◆), and of untreated A549 cells (○) and A549 cells treated with 10 nM Ara-C (●). Points are the average of quadruplicate haemocytometric cell counts from replicated experiments and the standard deviation never exceeded 5%. Cell viability, assessed by trypan blue analysis, was always higher than 90%

more rapid growth pattern of A549 cells, as compared to KB cells (doubling times of 24 h versus 36 h), could account for these apparent differences in cancer cell sensitivity to Ara-C. At these drug concentrations and exposure times, we were unable to detect changes in cell viability, as assessed by trypan blue dye exclusion and in growth recovery experiments, where cells exposed to Ara-C were allowed to grow after drug withdrawal (data not shown). The growth-inhibitory effect, which appeared to be cytostatic rather than cytotoxic, was time- and dose-dependent (Fig. 1 A, B).

Ara-C induces up-regulation of specific binding of ^{125}I-EGF and ^{125}I-transferrin on KB and A549 cells

An increased ^{125}I-EGF and ^{125}I-transferrin binding was found on KB and A549 cells after exposure to 1000 nM and 10 nM Ara-C respectively. The up-regulation occurred with a different timing for the two receptors on both cell lines. Maximal EGFR up-regulation was found after 96 h of Ara-C treatment while TrfR up-regulation peaked 24 h later (Fig. 2 A, B). To estimate the number of EGF- and transferrin-binding sites, increasing amounts of ^{125}I-EGF and ^{125}I-transferrin were added to KB cells and Scatchard analysis of binding data was performed at the times when EGFR and TfrR up-regulations were maximal. KB cells expressed EGF binding sites of low (K_d = 2.5 nM) and high (K_d = 0.05 nM) affinity and 96 h Ara-C treatment induced about a twofold increase in the number of both classes of EGFR (3.9×10^5 versus 6.3×10^5 and 3.1×10^4 versus 7.6×10^4, respectively) (Fig. 3 A, C). We found that Ara-C treatment did not affect EGFR and TrfR affinities for their ligands on KB cells (Fig. 3 A, B). A more than twofold increase of TrfR number (8.7×10^4 versus 21.3×10^4) was found on KB cells treated for 120 h with

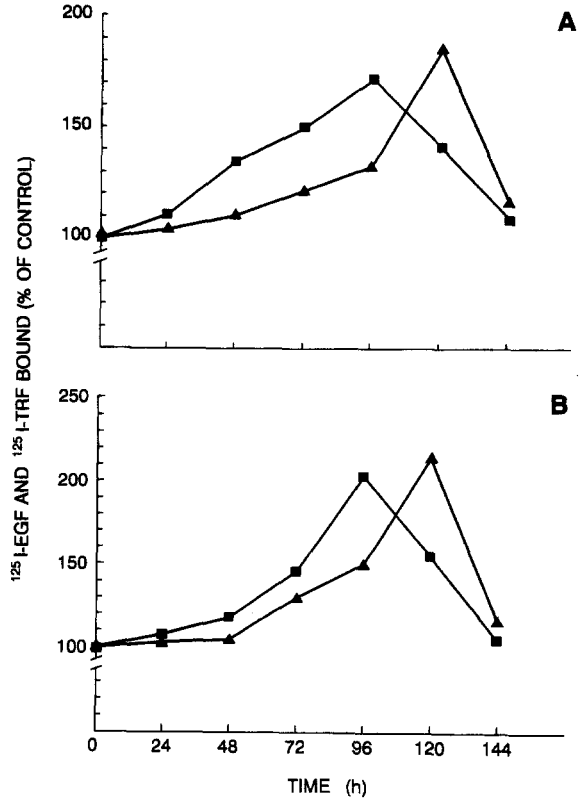

Fig. 2 A, B. Effects of Ara-C on epidermal growth factor (*EGF*) and transferrin (*TRF*) binding to KB and A549 cells. Specific binding of ^{125}I-EGF (■) and ^{125}I-transferrin (▲) to KB cells (**A**) and A549 cells (**B**) that were exposed to 1000 nM Ara-C and 10 nM Ara-C, respectively, was expressed as a percentage of results from parallel control cultures, which were not exposed to the drug. Non-specific binding was determined in the presence of a 100-fold excess of unlabelled EGF and transferrin respectively, and subtracted from each experimental point. The results in the figure have been derived from at least four different experiments. Points are the average of triplicate experiments and the standard deviation never exceeded 5%

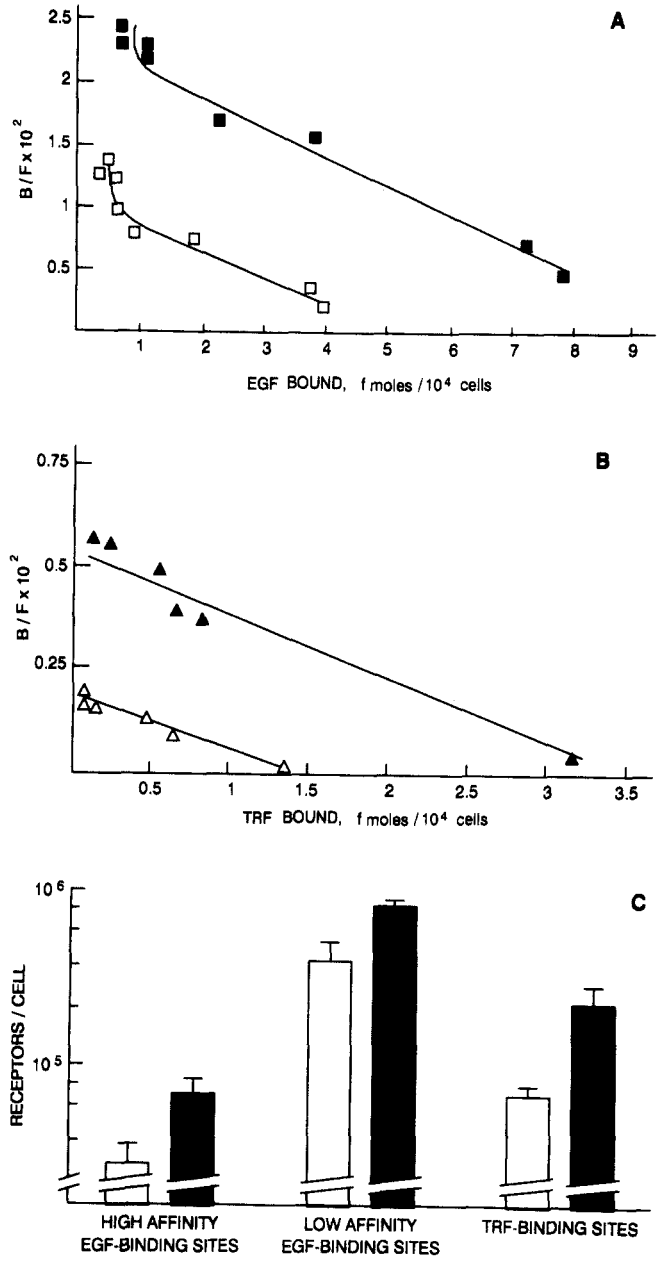

Fig. 3 A–C. Scatchard analysis of ^{125}I-EGF and ^{125}I-transferrin binding data on KB cells. **A** EGF binding was performed on untreated (□) and Ara-C-treated (■) KB cells after 96 h of exposure to the drug; **B** Transferrin (*TRF*) binding was evaluated on untreated (△) and Ara-C-treated (▲) KB cells after 120 h of exposure to the drug. At these times maximal receptor up-regulation is induced by 1000 nM Ara-C (See Fig 2 A). **C** Number of EGF and transferrin receptors per cell derived from Scatchard analysis. Untreated (*white columns*) and Ara-C-treated (*black columns*) KB cells. Data analysis from ^{125}I-EGF and ^{125}I-transferrin binding experiments was carried out with a curve-fitting (multiple-site) Scatchard analysis computer program. Results are the average of quadruplicate determinations, from at least four different experiments. Standard deviations were always lower than 5%

Ara-C and only one class of transferrin-binding sites (K_d = 4.1 nM) was detectable on these cells (Fig. 3 B, C).

An increase of EGFR and TrfR number, which occurred in the absence of changes of receptor affinity, was similarly induced by Ara-C on A549 cells (data not shown).

EGF and transferrin do not increase [^{3}H]thymidine uptake on Ara-C-treated KB cells

Since Ara-C treatment increases the expression of EGFR and TrfR, it can be hypothesized that an increased cell sensitivity to EGF/TGFα and transferrin could occur, so resulting in enhanced tumour proliferation and aggressiveness. Therefore, we have examined whether Ara-C treatment could induce an increased responsiveness of the KB cell line to EGF and transferrin stimulation of [^{3}H]thymidine uptake. EGF and transferrin induced an increase of [^{3}H]thymidine incorporation in KB cells, which is maximal after 12 h exposure to such growth factors, as evaluated in preliminary experiments (data not shown). Treatment of cells for 96 h and 120 h with 1000 nM Ara-C induced an approximately twofold increase of [^{3}H]thymidine uptake on KB cells. This effect could be the expression of S-phase synchronization of Ara-C-treated KB cells. The addition of EGF or transferrin (at 96 h and 120 h after the beginning of Ara-C treatment, respectively) did not cause any variation of [^{3}H]thymidine uptake on Ara-C-treated KB cells (Fig. 4).

Ara-C increases the binding of anti-EGFR and anti-TrfR monoclonal antibodies to KB and A549 cells

In order to evaluate whether the Ara-C-induced enhancement of ^{125}I-EGF and ^{125}I-transferrin binding was due to an increased number of EGFR and TrfR molecules that could be mAb-targeted at the tumour cell surface, we performed live-cell radioimmunoassay experiments with anti-EGFR mAb 108.1 and anti-TrfR mAb OKT9. These mAb recognize epitopes on the receptor molecules that are not located in the growth-factor-binding site [2, 36].

We found that an approximately twofold increase of both 108.1 (anti-EGFR) and OKT9 (anti-TrfR) binding to KB and A549 cells was actually induced by Ara-C on these cells (Fig. 5). The increase of mAb binding to Ara-C-treated KB and A549 cells paralleled, both in timing and magnitude, the up-regulation of ^{125}I-EGF and ^{125}I-transferrin binding.

Discussion

Selective delivery of anticancer drugs, radionuclides or bacterial toxins to the tumour tissues can be achieved through the use of anti-TAA immunoconjugates or through fusion proteins capable of binding to specific receptors at the tumour cell surface [10, 22, 27].

Cellular receptors for peptide growth factors are useful tools for cell targeting because they play a significant role in the regulation of tumour cell proliferation and behave as TAA [13, 16, 21, 24, 41]. In fact, anti-EGFR and anti-TrfR mAb are, at present, under investigation as anticancer agents [9, 14, 20]. In this respect, EGF or TGFα and the Fab′ fragment of anti-TrfR mAb fused to bacterial toxins are new promising reagents [6, 9, 20, 28].

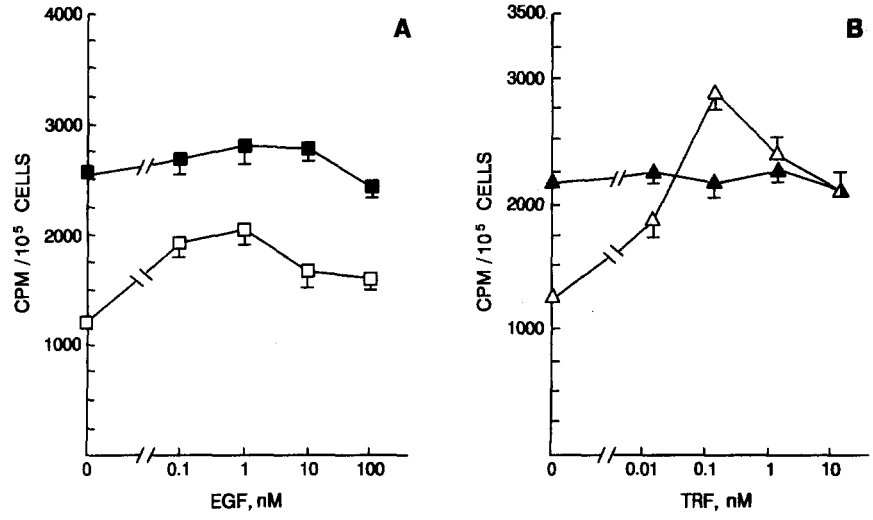

Fig. 4 A, B. [^{3}H]Thymidine uptake after exposure to EGF and transferrin (*TRF*) in untreated and Ara-C-treated KB cells. **A** [^{3}H]Thymidine uptake was evaluated on KB cells untreated (□) or treated for 96 h with 1000 nM Ara-C (■), after 12 h of exposure to different concentrations of EGF. **B** [^{3}H]Thymidine uptake was evaluated on KB cells untreated (△) or treated for 120 h with 1000 nM Ara-C (▲), after 12 h of exposure to different concentrations of transferrin. The results are the average of quadruplicate determinations evaluated at least in four different experiments. *Bars*, standard deviations

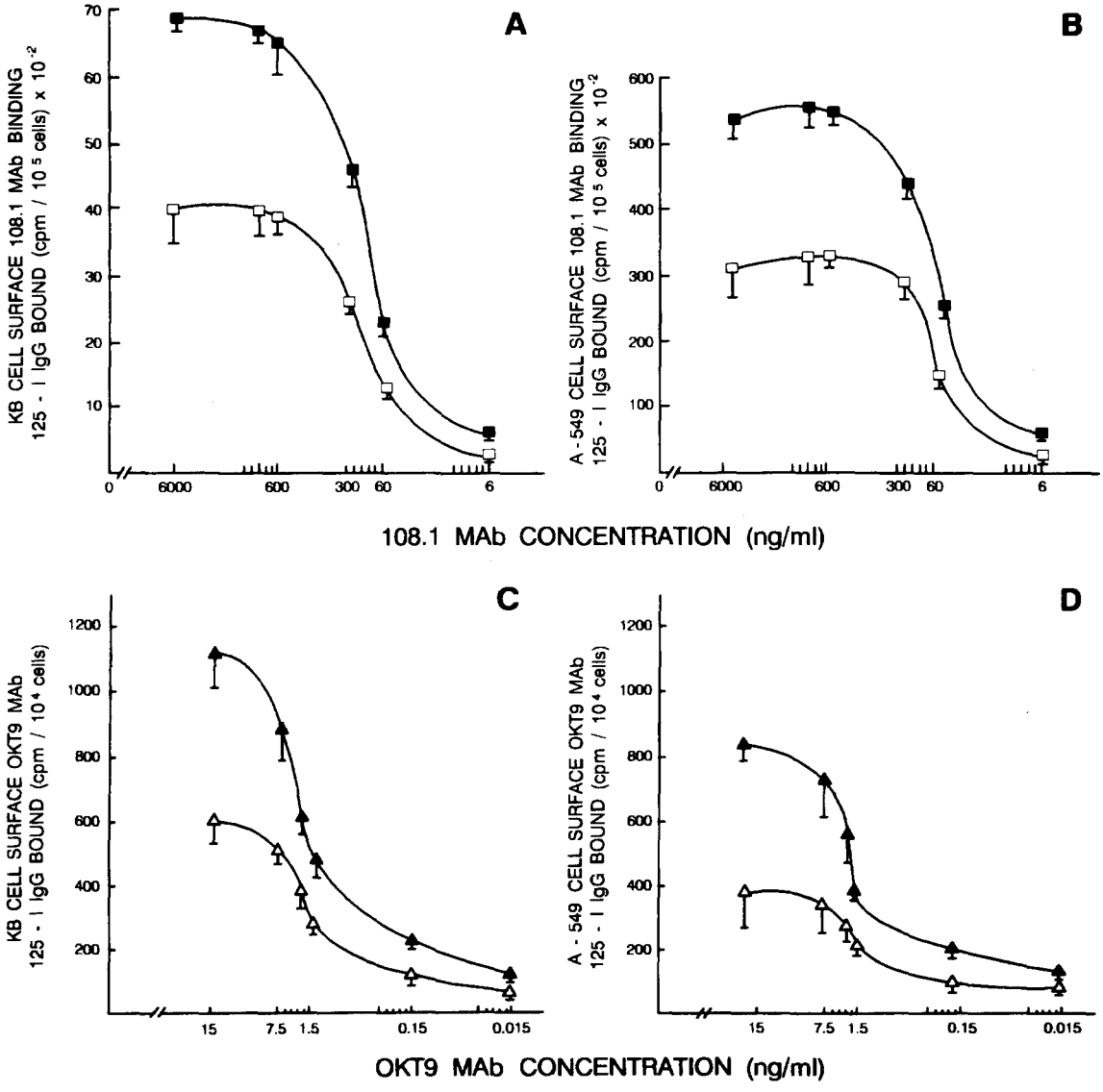

Fig. 5 A–D. Effects of Ara-C on the binding of anti EGF receptor (EGFR) and anti-transferrin receptor (TrfR) monoclonal antibodies to KB and A549 cells. **A, B** Binding of anti EGFR mAb 108.1 (**A**) to untreated KB cells (□) and KB cells treated with 1000 nM Ara-C for 96 h (■) and (**B**) to untreated A549 cells (□) and A549 cells treated with 10 nM Ara-C for 96 h (■). **C, D** Binding of anti-TrfR mAb (**C**) to untreated KB cells (△) and KB cells treated with 1000 nM Ara-C for 120 h (▲) and (**D**) to untreated A549 cells (△) and A549 cells treated with 10 nM Ara-C for 120 h (▲). The binding of anti-(growth factor receptor) mAb was assessed by live-cell radioimmunoassay, which was performed through the use of ^{125}I-labelled sheep anti-(mouse Ig) IgG and expressed as cpm/10^4 cells. The results are shown as the average of quadruplicate determinations evaluated at least in four different experiments. *Bars*, standard deviations

In this report we propose a new approach for the improvement of tumour cell targeting, which is founded on the pharmacological modulation of growth factor receptor density at the cancer cell surface. Several studies have, in fact, pointed to the density of TAA at tumour cell surface as one of the limiting factors for efficient targeting of tumour sites with anti-TAA mAb [14, 38]. It has been shown that in vivo targeting of human tumour cells with ^{111}In-labelled anti-EGFR mAb is correlated quantitatively with the number of membrane EGF receptor sites expressed by the cells [14]. Moreover, C. Sung et al. have reported that two mAb that recognise antigens expressed at a different density with similar affinity were rather different in the efficiency of in vivo tumour targeting: significantly higher tumour uptake could be observed with the mAb for the TAA that had an approximately threefold higher in vivo antigen density. These last results were obtained at mAb doses high enough to approach saturating conditions, more typical for therapeutic applications [38]. Furthermore, K. Fujimori et al. have recently proposed a mathematical model for the tissue distribution of the radioconjugated mAb: better tumour tissue percolation could be achieved for antibodies raised against antigens highly expressed at the tumour cell surface, while the use of mAb that have high affinity for less expressed antigens could lead to heterogeneous microdistribution and relatively higher mAb radiolocalization at the surface of tumour nodules (binding-site barrier) [11]. It could be suggested that efficient tumour cell targeting might be achieved through the use of agents able to increase the expression of TAA at the cancer cell surface, if in vitro and in vivo antigenic patterns are similar and the enhanced TAA expression does not increase the shedding of targeted antigens [3, 23, 29].

Interferons are widely considered as powerful modulators of antigen expression on the tumour cells and appear capable of also increasing the immunotargeting of tumour tissues [15, 31]. However, several tumour cells are not sensitive to the biological effects of IFN [43]. We have recently reported that cellular receptors for EGF could be up-regulated by IFNα on human epidermoidal carcinoma cells. We found that major up-regulation could be achieved at IFN concentrations capable of inducing 50% growth inhibition of such cells. We have proposed that EGFR modulation could be part of a homeostatic cell response to the anti-proliferative effects exerted by IFNα [4]. We have speculated that other agents, which could induce growth inhibition without affecting tumour cell viability, could also modulate the expression of EGFR. In fact, it could be of clinical benefit to increase EGFR and eventually TrfR expression with agents different from IFNα because several tumour cells are IFN-resistant [43]. For these purposes we selected Ara-C, which is known, at low doses, to determine growth inhibition without cell killing in myelodysplastic syndromes and acute leukaemias [12]. Moreover, this drug, at low doses, has antiproliferative activity and induces phenotypical changes on tumour cells [30].

Here we report that Ara-C induces growth inhibition and indeed up-regulates the EGF and transferrin binding to human oropharyngeal KB and lung A549 cancer cells, at doses that can be easily reached in vivo [18]. Ara-C exerts such effects on IFN-sensitive KB cells and on IFN-resistant A549 cells. The enhanced growth-factor binding induced by Ara-C is due to increased EGF and transferrin receptor number at the cancer cell surface and is paralleled by enhanced in vitro targeting of tumour cells with anti-EGFR and TrfR mAb. Both mAb 108.1 and OKT9 recognize epitopes on the receptor molecules distant from the growth-factor-binding site [2, 36]. Therefore, we argue for a true increase of membrane receptor proteins instead of the unmasking of receptor sites as the cause of the up-regulated growth factor binding induced by Ara-C.

The twofold increase of cell targeting with both 108.1 and OKT9 mAb, as induced by Ara-C on KB and A549 cells, resembles quantitatively the increase of antigen density induced by IFN on human tumour cells in culture [4, 15].

Receptor up-regulation was achieved in the absence of changes of the affinity for EGF and transferrin. This, therefore, allows tumour cell targeting with fusion proteins that bind to cellular receptors mimicking the physiological ligands.

The up-regulation of EGFR and TrfR and the concurrent increased binding of anti-(growth factor receptor) mAb occur with different timings, and the development of therapeutic strategies with the sequential administration of immunoconjugates and/or immunotoxins that bind the two different antigens can be considered. In fact, the heterogeneity in antigen expression and antigenic modulation by the cancer cells are mechanisms of tumour resistance to the antibody-targeted lysis [15, 25]. Up-regulation of different targets at different times from the beginning of Ara-C treatment, as described in this report, could allow a still more efficient targeting of the whole tumour cell population.

Ara-C produces, in growth-inhibited KB cells, an increase of [^{3}H]thymidine uptake that could be an expression of S-phase synchronization. In these conditions neither EGF nor transferrin could increase [^{3}H]thymidine uptake on these cells. Therefore we could assume that Ara-C-induced EGFR and TrfR up-regulation did not increase sensitivity of tumour cells to EGF and transferrin.

We conclude that Ara-C is a new agent that induces TAA modulation in cancer cells. From our results it can be suggested that EGFR and TrfR up-regulation induced by Ara-C could improve the specific targeting of tumour cells with mAb raised against growth factor receptors. Our observations could be of help for the design of sound protocols for in vivo studies of tumour cell targeting with anti-(growth factor receptor) mAb or growth-factor-receptor-targeted bacterial fusion proteins.

References

1. Aboud-Pirak AE, Hurwitz E, Pirak ME, Bellot F, Schlessingger J, Sela M (1988) Efficacy of antibodies to epidermal growth factor receptor against KB carcinoma in vitro and in nude mice. JNCI 80: 1605
2. Bellot F, Moolenaar W, Kris R, Mirakhur B, Verlaan I, Ullrich A, Schlessinger J, Felder S (1990) High affinity epidermal growth factor binding is specifically reduced by a monoclonal antibody, and appears necessary for early responses. J Cell Biol 110: 491

3. Boerman O, Massuger L, Makkink K, Thomas C, Kenemans P, Poels L (1990) Comparative in vitro binding characteristics and biodistribution in tumour-bearing athymic mice of anti-ovarian carcinoma monoclonal antibodies. Anticancer Res 10: 1289
4. Budillon A, Tagliaferri P, Caraglia M, Torrisi MR, Normanno N, Iacobelli S, Palmieri G, Stoppelli MP, Frati L, Bianco AR (1991) Upregulation of epidermal growth factor receptor induced by alpha-interferon in human epidermoid cancer cells. Cancer Res 51: 1294
5. Cross M, Dexter TM (1991) Growth factors in development, transformation, and tumorigenesis. Cell 64: 271
6. Debinski W, Fitzgerald D, Pastan I (1992) An active monovalent immunotoxin composed of *Pseudomonas* exotoxin coupled to the Fab′ fragment of an antibody to the transferrin receptor (abstract). Proc Am Assoc Cancer Res 33: 343
7. De Larco JE, Todaro GJ (1978) Growth factor from murine sarcoma virus-transformed cells. Proc Natl Acad Sci USA 75: 4001
8. Dickson RB, Hanover JA, Willingham MC, Pastan I (1983) Prelysosomal divergence of transferrin and epidermal growth factor during receptor-mediated endocytosis. Biochemistry 22: 5667
9. Dvigi CR, Welt S, Kris M, Real FX, Yeh SDJ, Gralla R, Merchant B, Schweighart S, Unger M, Larson SM, Mendelsohn J (1991) Phase I and imaging trial of indium 111-labeled anti-epidermal growth factor receptor monoclonal antibody 225 in patients with squamous cell lung carcinoma. JNCI 83: 97
10. Foon KA (1989) Biological response modifiers: the new immunotherapy. Cancer Res 49: 1621
11. Fujimori K, Fisher DR, Winstein JN (1991) Integrated microscopic-macroscopic pharmacology of monoclonal antibody radioconjugates: the radiation dose distribution. Cancer Res 51: 4821
12. Gale RP, Foon KA (1987) Therapy of acute myelogenous leukemia. Semin Hematol 24: 40
13. Gatter KC, Brown G, Trowbridge IS, Woolston R-E, Mason DY (1983) Transferrin receptors in human tissues: their distribution and possible clinical relevance. J Clin Pathol 36: 539
14. Goldenberg A, Masui H, Divgi C, Kamrath H, Pentlow K, Mendelsohn J (1989) EGF receptor overexpression and localization of nude mouse xenografts using an indium-111-labeled anti-EGF receptor monoclonal antibody. JNCI 81: 1616
15. Greiner JW, Horan Hand P, Noguchi P, Fisher PB, Pestka S, Schlom J (1984) Enhanced expression of surface tumour-associated antigens on human breast and colon tumour cells after recombinant human leukocyte alpha-interferon treatment. Cancer Res 44: 3208
16. Gullick WJ, Marsden JJ, Whittle N, Ward B, Bobrow L, Waterfield MD (1986) Expression of epidermal growth factor receptors on human cervical, ovarian and vulval carcinomas. Cancer Res 46: 285
17. Haeder M, Rotsch M, Bepler G, Henning C, Havemann K, Heimann B, Moelling K (1983) Epidermal growth factor receptor expression in human lung cancer cell lines. Cancer Res 48: 1132
18. Ho DHW, Frei E III (1971) Clinical pharmacology of 1-beta-D-arabinofuranosylcytosine. Clin Pharmacol Ther 12: 944
19. Jetten AM (1980) Retinoids specifically enhance the number of epidermal growth factor receptors Nature 284: 626
20. Johnson VG, Wrobel C, Wilson D, Zovickian J, Greenfield L, Oldfield EH, Youle R (1989) Improved tumour-specific immunotoxins in the treatment of CNS and leptomeningeal neoplasia. J Neurosurg 70: 240
21. Liebermann TA, Razon N, Bartal AD, Yarden Y, Schlessinger J, Soreq H (1984) Expression of epidermal growth factor receptors in human brain tumours. Cancer Res 44: 753
22. Maraveyas A, Epenetos AA (1991) An overview of radioimmunotherapy. Cancer Immunol Immunother 34: 71
23. Matzku S, Bruggen J, Brocker E-B, Sorg C (1987) Criteria for selecting monoclonal antibodies with respect to accumulation in melanoma tissue. Cancer Immunol Immunother 24: 151
24. Mendelsohn J (1988) Growth factor receptors as targets for antitumour therapy with monoclonal antibodies. Prog Allergy 45: 147
25. Miller RA, Oseroff AR, Stratte PT, Levy R (1983) Monoclonal antibody therapeutic trials in seven patients with T-cell-lymphoma. Blood 62: 988
26. Munson PJ, Rodbard D (1980) LIGAND: a versatile computerized approach for characterization of ligand binding systems. Anal Biochem 107: 220
27. Neckers LM, Cossman J (1983) Transferrin receptor induction in mitogen-stimulated human T lymphocytes is required for DNA synthesis and cell division and is regulated by interleukin 2. Proc Natl Acad Sci USA 80: 3494
28. Pastan I, Fitzgerald DJ (1991) Recombinant toxins for cancer treatment. Science 254: 1173
29. Philben VJ, Jakowatz JG, Beatty BG, Vlahos WG, Paxton RJ, Williams LE, Shiveley JE, Beatty JD (1986) The effect of tumour CEA content and tumour sites on tissue uptake of indium 111-labeled anti-CEA monoclonal antibody. Cancer 57: 571
30. Pinto A, Attadia V, Rosati R, Colletta G, Cimino R, Colombatti R (1988) Differentiation of human leukemic cell lines and fresh leukemia cells by low dose ara-C: monitoring by expression of c-*myc* and c-*fos* oncogenes. Med Oncol Tumor Pharmacother 5: 91
31. Rosemblum MG, Lamki LM, Murray JL, Carlo DJ, Gutterman JV (1988) Interferon-induced changes in pharmacokinetics and tumour uptake of ^{111}In-labelled antimelanoma antibody 96.5 in melanoma patients. JNCI 80: 160
32. Ruff E, Rizzino A (1986) Preparation and binding of radioactively labeled porcine transforming growth factor type beta. Biochem Biophys Res Commun 138: 714
33. Scatchard G (1949) The attraction of proteins for small molecules and ions. Ann NY Acad Sci 51: 600
34. Schlessinger J (1986) Allosteric regulation of epidermal growth factor receptor kinase. J Cell Biol 103: 2067
35. Schlessinger J, Schreiber AB, Levi A, Lax I, Liberman T, Yarden Y (1983) Regulation of cell proliferation by epidermal growth factor. CRC Crit Rev Biochem 16: 93
36. Schneider C, Sutherland R, Newman R, Greaves M (1982) Structural features of the cell surface receptor for transferrin that is recognized by the monoclonal antibody OKT9. J Biol Chem 257: 8516
37. Sung C, Youle RJ, Dedrick RL (1990) Pharmacokinetic analysis of immunotoxin uptake in solid tumors: role of plasma kinetics, capillary permeability, and binding. Cancer Res 50: 7382
38. Sung C, Shockley Ty R, Morrison PF, Dvorak HF, Yarmush ML, Dedrick RL (1992) Predicted and observed effects of antibody affinity and antigen density on monoclonal antibody uptake in solid tumors. Cancer Res 52: 377
39. Taetle R, Honeysett JM, Bergeron R (1989) Combination iron depletion therapy. JNCI 81: 1229
40. Toi M, Nakamura T, Mukaida H, Wada T, Osaki A, Yamada H, Toge T, Niimoto M, Hattori T (1990) Relationship between epidermal growth factor status and various prognostic factors in human breast cancer. Cancer 65: 1980
41. Trowbridge IS (1988) Transferrin receptor as a potential therapeutic target. Prog Allergy 45: 128
42. Trowbridge IS, Shackelford DA (1985) Structure and function of transferrin receptors and their relationship to cell growth. Biochem Soc Symp 51: 117
43. Vignaux F, Knight E Jr, Eid P, Gresser I (1991) An interferon-induced 16-KD protein is present in the membranes of interferon-sensitive but not in interferon-resistant mouse cells. J Interferon Res 11: 123