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RESEARCH PAPER

Carcinoembryonic antigen-stimulated THP-1 macrophages activate endothelial cells and increase cell–cell adhesion of colorectal cancer cells

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Abstract The liver is the most common site for metastasis by colorectal cancer, and numerous studies have shown a relationship between serum carcinoembryonic antigen (CEA) levels and metastasis to this site. CEA activates hepatic macrophages or Kupffer cells via binding to the CEA receptor (CEA-R), which results in the production of cytokines and the up-regulation of endothelial adhesion molecules, both of which are implicated in hepatic metastasis. Since tissue macrophages implicated in the metastatic process can often be difficult to isolate, the aim of this study was to develop an in vitro model system to study the complex mechanisms of CEA-induced macrophage activation and metastasis. Undifferentiated, human monocytic THP-1 (U-THP) cells were differentiated (D-THP) to macrophages by exposure to 200 ng/ml phorbol myristate acetate (PMA) for 18 h. Immunohistochemistry showed two CEA-R isoforms present in both U- and D-THP cells. The receptors were localized primarily to the nucleus in U-THP cells, while a significant cell-surface presence was observed following PMA-differentiation. Incubation of D-THP-1 cells with CEA resulted in a significant increase in tumor necrosis factor-alpha (TNF-α) release over 24 h compared to untreated D-THP-1 or U-THP controls confirming the functionality of these cell surface receptors. U-THP cells were unresponsive to CEA. Attachment of HT-29 cells to human umbilical vein endothelial cells significantly increased at 1 h after incubation with both recombinant TNF-α and conditioned media from CEA stimulated D-THP cells by six and eightfold, respectively. This study establishes an in vitro system utilizing a human macrophage cell line expressing functional CEA-Rs to study activation and signaling mechanisms of CEA that facilitate tumor cell attachment to activated endothelial cells. Utilization of this in vitro system may lead to a more complete understanding of the expression and function of CEA-R and facilitate the design of anti-CEA-R therapeutic modalities that may significantly diminish the metastatic potential of CEA overexpressing colorectal tumors.

Keywords Carcinoembryonic antigen · Carcinoembryonic antigen receptors · Colon cancer · Metastasis · THP-1 macrophages

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Introduction

Several lines of evidence support the concept that tumor-associated macrophages facilitate tumor progression and metastasis; however, the mechanisms are not well defined [1]. It has been proposed that macrophages may promote tumor extravasation, growth and angiogenesis through the production of cytokines, growth factors, and angiogenic inducers, such as epidermal growth factor, vascular endo-

thelial growth factor, and tumor necrosis factor-alpha (TNF-α) [2–5].

Liver macrophages or Kupffer cells are perhaps one of the better examples of the complex relationship that tumor-associated macrophages can play in facilitating the metastatic process. These tissue macrophages are located within the sinusoids of the liver and represent the largest population of fixed macrophages in the human body. Kupffer cells can secrete potent mediators of the inflammatory response (e.g., TNF-α and other cytokines, reactive oxygen species, eicosanoids, nitric oxide, and carbon monoxide) which can up-regulate NF-κB activity in both target tissue cells and in macrophages themselves, and thus mediate the early extravasation phase of tumor cells [6, 7].

The liver is the most common site of metastasis by colorectal cancers [8]. Numerous studies have described the complex function that hepatic macrophages play in the progression of colon cancer to the liver [9]. Although substantial gaps in our knowledge still remain, the role of carcinoembryonic antigen (CEA) in the progression of colon cancer cells to the liver is more firmly established [10–13]. CEA is a glycoprotein of ~180 kDa that is frequently overexpressed by colorectal tumors [14, 15]. While it is not an effective marker for screening, CEA is the most useful indicator for monitoring the therapeutic efficacy of surgery in colorectal cancer, as increasing levels of CEA in the serum after surgery often correlate with either local recurrence or the development of metastasis [16, 17]. Kupffer cells clear CEA from the circulation via the CEA receptor (CEA-R), an ortholog of the human heterogeneous nuclear RNA binding protein (hnRNP) M4 [18]. The CEA-R consists of several splice variants, including the full-length protein (long, CEAR-L) and a form with a 39 amino-acid deletion between RNA binding domains one and two (short, CEAR-S) [19–21].

The cytokines produced by CEA activated Kupffer cells, especially interleukin (IL) 1β, IL-6, and IL-10, can both protect against cytotoxicty and facilitate the adhesion of metastatic tumor cells to the hepatic sinusoidal endothelium [22, 23]. In addition, cytokine-driven activation of endothelial cells results in the up-regulation of cell surface adhesion molecules, which in turn facilitate the arrest and extravasation of circulating tumor cells [7, 24–26]. This gives CEA-producing colorectal cancer cells a selective advantage for uptake by the liver, which increases their metastatic potential. Although these studies suggest that CEA plays a central role in the formation of hepatic metastasis of colorectal tumors, the signaling mechanisms by which CEA activates Kupffer cells, and subsequently, endothelial cells have yet to be fully understood. Since the isolation of primary Kupffer cells for experimental studies is often an arduous and time-consuming process, a human macrophage cell line that expresses functional CEA-Rs may offer a surrogate model to study CEA-signaling mechanisms related to colorectal cancer metastasis. In this communication, we describe an in vitro system utilizing differentiated THP-1 (D-THP) cells with functional CEA-R that may be a useful model for the study of CEA-related signaling and metastatic events.

Materials and methods

All chemicals were obtained from Sigma unless otherwise noted. The CEA used in these experiments was purified from hepatic colorectal cancer metastasis as previously described [27]. All cells were obtained from the American Type Culture Collection (Manassas, VA, USA) and were cultured at 37°C in 5% CO_2, 95% air. Cell culture media, supplements, and antibiotics were obtained from GibcoBRL/Life Technologies Inc., Carlsbad, CA, USA and fetal bovine serum was obtained from Cellgro, Hendon, VA, USA.

Cell culture

The human monocyte cell line, THP-1, was grown in RPMI medium, containing 10% fetal bovine serum, 2 mM L-glutamine, 10,000 U/ml penicillin G, 25 μg/ml amphotericin B, and 10,000 μg/ml streptomycin, which was replaced every 2–3 days. Prior to experiments, THP-1 monocytes in suspension were differentiated to attached macrophages by exposure to phorbol myristate acetate (PMA) (200 ng/ml) for 18 h. After differentiation, the attached cells were at confluency and were washed three times in complete media and were incubated for 24 h prior to usage. Human umbilical vein endothelial cells (HUVECs) were cultured in Ham's F-12K medium with L-glutamine and sodium bicarbonate supplemented with 10% fetal bovine serum, 0.1 mg/ml heparin, 10,000 U/ml penicillin G, 25 μg/ml amphotericin B, and 10,000 μg/ml streptomycin. HUVECs were grown to confluence in 100 mm plates and then seeded onto 96-well plates. HT-29 colorectal cancer cells were cultured in Dulbecco's Modified Eagle Medium, high glucose with L-glutamine and sodium pyruvate supplemented with 10% fetal bovine serum, 10,000 U/ml penicillin G, 25 μg/ml amphotericin B, and 10,000 μg/ml streptomycin.

Detection of CEA-receptors by immunohistochemistry

Polyclonal antibodies specific to the short and long splice variants of the CEA-R, CEAR-S or CEAR-L, were developed with synthetic antigenic peptides, as previously described [20] and were used to identify the cellular location of the CEA-R in undifferentiated (U-THP) and PMA-D-THP cells as previously described [20].

CEA-binding studies

Carcinoembryonic antigen binding to U-THP and D-THP cells was performed, as previously described [19]. Briefly, CEA was radio labeled with ^{125}I by the chloramine-T method [28]. ^{125}I-CEA (5 μg/ml) was incubated with the U-THP and D-THP cells at 4 and 37°C in a binding buffer consisting of glucose (6 mg/ml), bovine serum albumin (0.6 mg/ml), sodium chloride (120 mM), potassium chloride (5 mM), magnesium chloride (5 mM), calcium chloride (1 mM), and HEPES buffer (20 mM). Cells were harvested at 5, 15, 30, and 60 min intervals and were immediately centrifuged at 14,000 rpm through an oil separation suspension consisting of dibutylphthalate : dioctylphthalate (3:1). The cells, once spun through the oil and pelleted, were separated from the supernatant for gamma counting by cutting off the bottom of the centrifuge tube and collecting the cell pellet. Specificity of CEA binding was tested by incubating D-THP cells with ^{125}I-CEA, as described above, in the presence of a 50-fold excess of unlabeled CEA.

Measurement of cytokine production by undifferentiated and differentiated THP-1 cells

The U-THP and D-THP cells were incubated at 37°C in the presence or absence of CEA (2 μg/ml) for up to 24 h in 6-well plates with 1 ml of RPMI media containing 0.5% fetal bovine serum. Media was collected at 2, 4, 6, 12, and 24 h, centrifuged to remove cells and/or debris and frozen at –80°C until analysis. TNF-α levels were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kits (Cayman Chemicals, Ann Arbor, MI, USA). To validate the specificity of CEA for the CEA-R, antibody blocking experiments were conducted with D-THP cells which were again treated with CEA (2 μg/ml) in the presence or absence of a specific, polyclonal CEA-R antibody that was generated to a common sequence of the short and long forms of the receptor and thus recognizes both. Production and characterization of the CEA-R specific antibody has been previously described [20]. Since D-THP cells are known to be very responsive to lipopolysaccharide (LPS) [29], we further assessed functionality by incubating D-THP cells for 4 h with LPS (10 ng/ml). Media was collected to measure TNF-α production as described above.

Measurement of endothelial adhesion molecule cell surface and mRNA expression

The endothelial cell-surface expression of the adhesion molecules, intercellular adhesion molecule-1 (ICAM-1), and E-selectin, were measured using a modified ELISA protocol as previously described [30–33]. Briefly, HUVECs were plated equally on 96-well, flat-bottom plates and grown to confluence at 37°C as described above. Prior to the experiment, the cells were washed with warm media and stimulated for 24 h with TNF-α (10 ng/ml). After stimulation, the endothelial cell monolayers were washed with PBS and fixed with 2% paraformaldehyde. Prior to incubation with antibodies, cells were briefly washed with 5% non-fat dry milk to reduce non-specific binding. Cells were then incubated with primary antibody ICAM-1 (1 μg/ml) and E-selectin (2 μg/ml) (R&D Systems, Minneapolis, MN, USA) overnight, after which the cells were washed with PBS and incubated for 1 h at room temperature with a peroxidase-conjugated horse anti-mouse IgG (Cell Signaling Technology, Beverly, MA, USA). After washing again, the substrate 3,3′, 5,5′-tetramethylbenzidine (TMB) was added for 30 min, after which its action was quenched with 1.6 N sulfuric acid. Optical density was determined at 450 nm using a spectrophotometer (SpectroMax 250, Molecular Devices, Sunnyvale, CA, USA).

Total RNA was isolated from the cells with the SV Total RNA Isolation System (Promega, Madison, WI, USA) and semi-quantitative PCR was performed (Gene Amp PCR System 2400 from Applied Biosystems, Foster City, CA, USA). The following primer sets were used to amplify ICAM-1 and E-selectin: ICAM-1, 5′-CAGTGACCATCTACAGCTTTCCGG-3′ (sense) and 5′-GCTGCTACCACAGTGATGATGACAA-3′ (antisense); E-selectin, 5′-CTCTGACAGAAGAAGCCAAG-3′ (sense) and 5′-ACTTGAGTCCACTGAAGCCA-3′ (antisense).

Quantification of the attachment of HT-29 cell to endothelial cells

Human umbilical vein endothelial cells were seeded into 96-well plates and incubated at 37°C to a confluent monolayer as described above. Prior to experiments, HUVECs were stimulated with either recombinant human TNF-α (10 ng/ml) or conditioned media from D-THP cells incubated with CEA (2 μg/ml) for 24 h. HT-29 cells were fluorescently labeled with 1,1′-dioctadecyl-3, 3, 3′, 3′-tetramethylindocarbocyanine (DiI) as described by Hiscox and Jiang [34]. Unlabeled or labeled cells were added to the HUVEC-containing plates at a concentration of 2×10^5 cells/well and the cells were co-cultured for 30 min intervals for up to 90 min. After washing the unattached cells off the plates twice with PBS, the plates were then read with a fluorescent plate reader (Gemini XPS from Molecular Devices Corp.) with excitation set at 540 nm and emission set at 590 nm [34].

Statistical analysis

All data are expressed as means ± SEM and were analyzed by one-way analysis of variance with SigmaStat statistical software (Systat Software, Point Richmond, CA, USA). When significant ($P < 0.05$), the difference between specific means was determined using the Student–Newman–Keuls test.

Results

CEA Receptors translocate to the cell surface of THP-1 cells after differentiation

Immunohistochemistry demonstrated that both the CEAR-S and CEAR-L splice variants of the CEA-R were located primarily in the nuclei and the cytoplasm of U-THP cells (Fig. 1a). These cells grew well and typified monocytes in suspension. However, once the cells were differentiated overnight by exposure to PMA, they attached to the culture plates and demonstrated phenotypic characteristics of confluent macrophages. A large percentage of the receptors were uniformly translocated to the cell surface and was most pronounced with the CEAR-S (Fig. 1b).

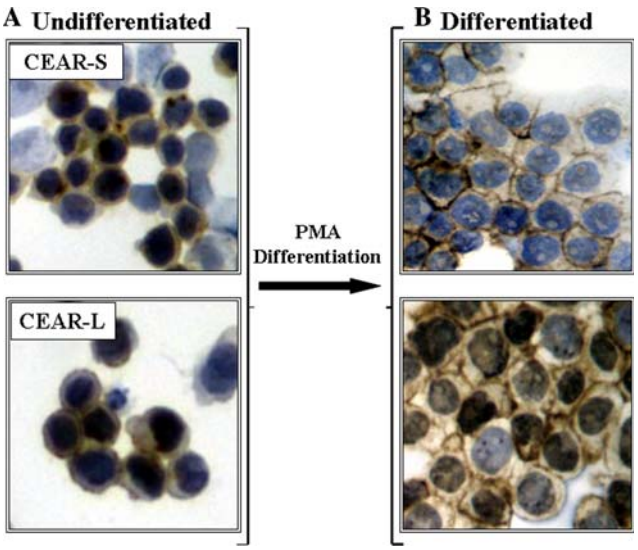

Fig. 1 CEA receptor localization in undifferentiated and PMA-differentiated THP-1 cells. (**a**) Both short and long forms of the CEA receptor are localized primarily to the nucleus in undifferentiated cells. (**b**) The receptors were translocated to the cell surface upon differentiation by 200 ng/ml of PMA for 18 h. *CEA* carcinoembryonic antigen, *PMA* phorbol myristate acetate

Radiolabeled CEA binds specifically to differentiated THP-1 cells

^{125}I-labeled CEA was used to assess the specificity of the interaction of CEA with both U- and D-THP cells. These ligand-binding studies demonstrated that there was significantly ($P < 0.05$) greater binding of ^{125}I-labeled CEA to D-THP cells compared to U-THP cells controls in a time-dependent fashion (up to 1 h) (Fig. 2). These findings were similar in experiments conducted on D-THP cells that were incubated at 4°C to minimize internalization of the CEA-R complex and at 37°C. At 60 min, D-THP cells showed a 3.9-fold and a 3.4-fold increase in CEA binding at these respective temperatures compared to controls. A 50-fold excess of unlabeled CEA competitively inhibited binding to the receptor in D-THP cells and resulted in a 63 and 59% decrease at 4 and 37°C, respectively, at 1 h suggesting that the interaction of CEA with the CEA-R is specific.

CEA increases TNF-α production in differentiated THP-1 cells

Even though the translocated CEA-Rs specifically bound radiolabeled CEA, we next sought to ascertain the functionality of the cell-surface CEA-Rs. U- and D-THP cells were incubated with CEA (2 μg/ml) and the production of the proinflammatory cytokine TNF-α was measured in the media by ELISA. By 4 h, TNF-α levels rose by 69% in CEA-treated D-THP cells ($P < 0.05$) compared with untreated D-THP cells and remained significantly elevated for up to 24 h (Fig. 3a). CEA treated U-THP cells were significantly less responsive to CEA compared with either untreated or CEA treated D-THP cells. However, the U-THP cells may have some functional cell surface receptors since there was a small, but not statistically significant, response to CEA.

To validate the specificity of the CEA binding to its receptor, antibody blocking experiments were conducted in D-THP cells with a specific CEA-R antibody that recognizes the short and long forms of the receptor. These experiments showed that the CEA-R antibody effectively reduced the CEA mediated TNF-α response at 4 and 8 h ($P < 0.05$) (Fig. 3b). By 12 h, receptor recycling and diminished antibody concentrations in the media reduced the effectiveness on the inhibition, which was completely ablated by 24 h.

Even though D-THP cells have been shown to secrete copious amounts of TNF-α in response to LPS stimulation compared to U-THP cells [29], one final set of experiments was conducted to determine the responsiveness of D-THP cells to LPS compared with CEA. Interestingly, 1 ng/ml of ultrapure LPS elicited nearly the same TNF-α response as 2 μg/ml of CEA in D-THP cells, 920 pg/ml vs. 900 pg/ml,

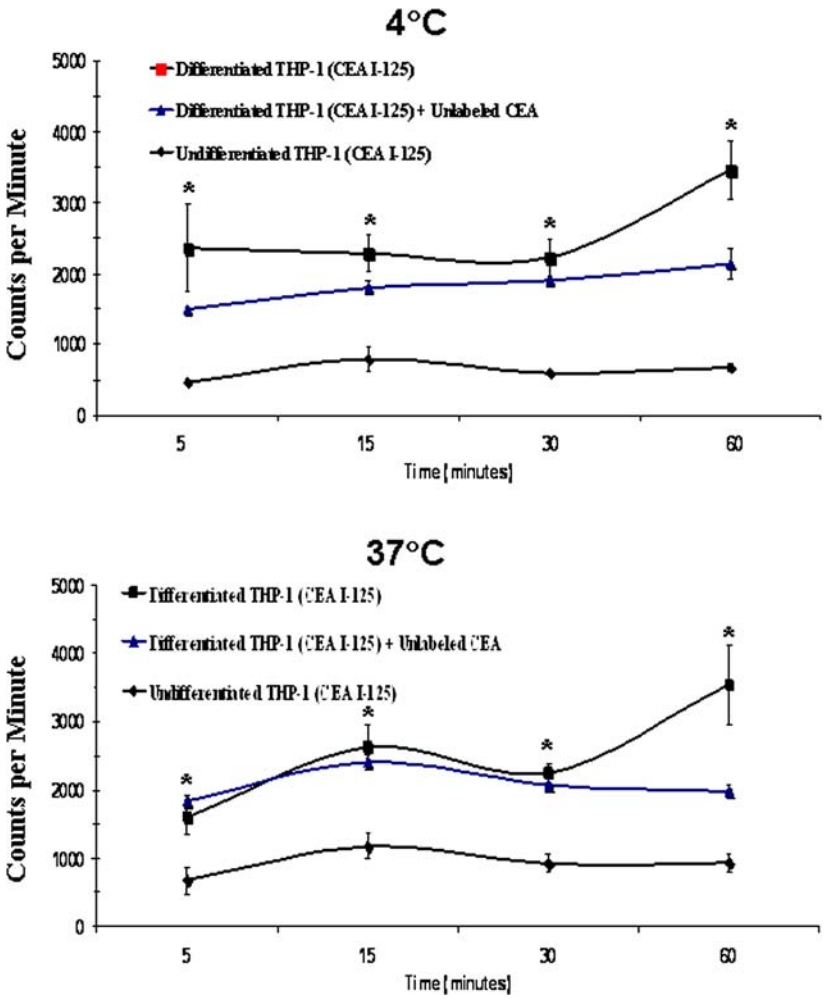

Fig. 2 Radio labeled CEA binding to undifferentiated and differentiated THP-1 cells. Binding of ^{125}I-CEA to differentiated THP-1 cells was significantly increased ($P < 0.05$) compared to undifferentiated THP-1 controls at all time points at both 4°C (*Top*) and 37°C (*Bottom*). Binding specificity was demonstrated by showing that an excess of unlabeled, or cold, CEA could competitively inhibit ligand binding, especially at 60 min. Results are shown as means ± SEM. $n = 3$ in triplicate. *CEA* carcinoembryonic antigen * $P < 0.05$ compared to U-THP-1 cells

respectively, whereas untreated D-THP cells produced ~53 pg/ml, further indicating that the D-THP cells used in the subsequent HT-29 colorectal cancer cell binding studies retain their inherent capacity to synthesize cytokines. Shiratsuch and Basson [29] also showed that U-THP cells were relatively unresponsive to LPS compared with D-THP cells (530 pg/ml vs. 37,387 pg/ml), respectively. As mentioned previously, the up-regulation of the proinflammatory cytokine, TNF-α, from macrophages is of particular importance in this model because macrophages have been implicated in the mechanism of tumorigenesis and metastasis of colorectal cancer cells through the modulation of adhesion molecules on the endothelium.

TNF-α activation of endothelial cells increases the mRNA and cell-surface expression of the adhesion molecules E-selectin and ICAM

The attachment of circulating tumor cells to the vascular endothelium is facilitated by the up-regulation of endothelial adhesion molecules and is a key pathophysiologic event that leads to metastasis. It has been proposed that this event is exacerbated by inflammatory cytokines such as TNF-α produced by tissue macrophages. This study demonstrated significant increases in both the mRNA and cell-surface expression of the adhesion molecules E-selectin and ICAM-1 in response to a 24 h treatment of HUVECs with human recombinant TNF-α (10 ng/ml). Semi-quantitative PCR showed that treatment with TNF-α increased the mRNA expression of ICAM-1 and E-selectin 645 and 72%, respectively, compared with untreated controls (Fig. 4a). The TNF-α-mediated increases in mRNA expression translated into 766 and 1,151% increases in cell-surface expression of E-selectin and ICAM-1, respectively, compared to untreated controls as measured by ELISA (Fig. 4b).

Activation of endothelial cells by conditioned media from CEA-stimulated differentiated THP-1 cells increases the attachment of HT-29 human colon cancer cells

Given that the proinflammatory cytokine TNF-α, secreted in high amounts from CEA-stimulated D-THP cells,

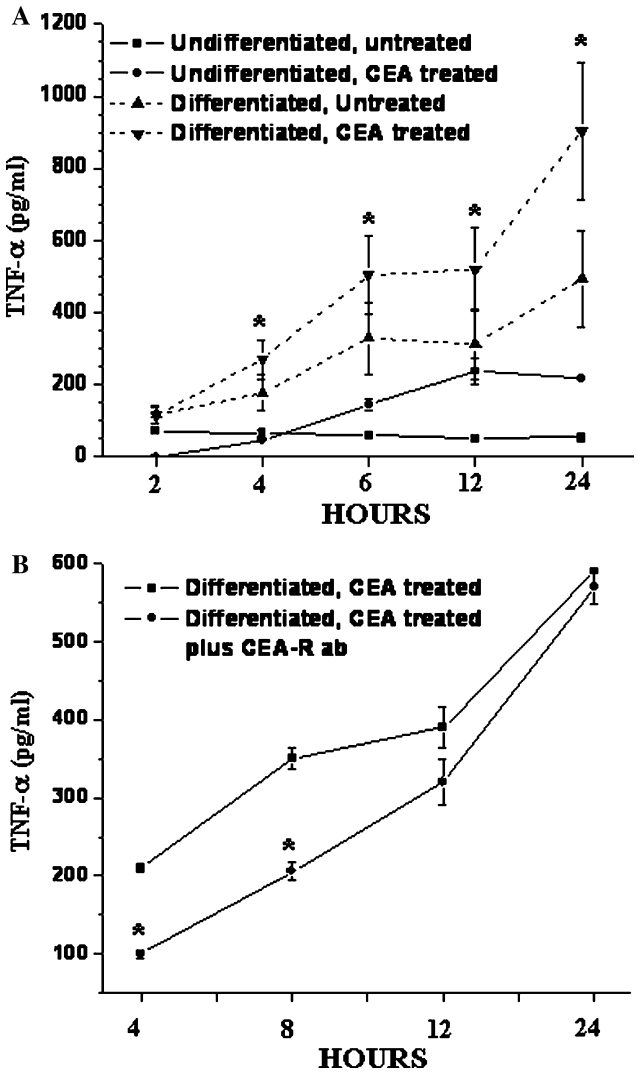

Fig. 3 TNF-α production in untreated and CEA stimulated, undifferentiated and differentiated THP-1 cells. (**a**) CEA significantly increased TNF-α production in differentiated THP-1 cells ($n = 8$) compared to undifferentiated, CEA treated cells ($n = 8$) at all time points and at 24 h compared with differentiated, untreated THP-1 cells ($n = 8$). **b** The effect of a CEA receptor antibody on TNF-α production in CEA stimulated differentiated THP-1 Cells. A specific CEA-R antibody (*CEA-R ab*) that recognized both the long and short splice variants of the receptor significantly reduced the CEA mediated TNF-α response at 4 and 8 h * ($P < 0.05$) compared with CEA treated cells alone. By 12 h, receptor recycling and antibody concentrations in the media began to diminish the effectiveness on the inhibition, which was completely ablated by 24 h. Results are shown as means ± SEM at each time point. * $P < 0.05$ compared to respective controls. *CEA* carcinoembryonic antigen, *CEA-R* CEA receptor, *TNF-α* tumor necrosis factor alpha

increases the mRNA and cell-surface expression of endothelial adhesion molecules, it was our hypothesis that the attachment of HT-29 cells to HUVECs would increase dramatically when incubated with conditioned media from D-THP cells incubated with CEA. Recombinant human TNF-α was used as a positive control. We show that HT-29 attachment to HUVECs treated with the conditioned media from the D-THP cells was significantly ($P < 0.05$) increased at 60 and 90 min compared with TNF-α treated and untreated controls (Fig. 5).

Discussion

A well characterized in vitro system can be invaluable to our understanding of metastatic pathways, cellular interactions at the primary site and complex signaling mechanisms that mediate metastasis. Since key cell types implicated in the metastatic processes in the liver, such as Kupffer cells, can be difficult to isolate in vivo, a macrophage cell line that responds to CEA can be a very useful research tool. In this study, we showed that D-THP macrophages express functional cell-surface CEA-Rs that specifically bind CEA, and in response, secrete significant quantities of TNF-α. In addition, incubation of HUVECs with conditioned media from CEA-stimulated D-THP cells subsequently induced the cell-surface expression of the adhesion molecules ICAM-1 and E-selectin, and the co-incubation of HT-29 colorectal cancer cells in this system recapitulated the increased attachment of colorectal cancer cells in vivo. These results validate this in vitro model as a means of studying the mechanisms by which CEA modulates the hepatic sinusoidal microenvironment and facilitates colorectal cancer metastasis.

We also demonstrated that the CEA-Rs that translocated from the nucleus and cytoplasm to the cell surface are functional in that there was significantly more binding of radio labeled CEA to the D-THP cells, which ultimately resulted in significantly increased TNF-α production. This is a new finding and of particular relevance because previous work with other macrophage cell lines (CRL 2192, P338D1, IC-21, and Raw 246) have shown variable localization of the CEA-R and P338D1 was the only cell line that was able to bind CEA, but only after being transfected with cDNA of the CEA-R [19].

Metastatic disease is the principal cause of death for the majority of colorectal cancer patients [35]. The metastasis of colorectal cancers to the liver is a complex process that involves multiple cell types including hepatic sinusoidal Kupffer cells, endothelial cells, and the metastasizing tumor cells themselves [7]. Kupffer cells are thought to play a central role in the development of hepatic metastasis from CEA overexpressing cancers [12]. In a working model depicting the activation of Kupffer cells by CEA (Fig. 6), CEA produced by colorectal cancer cells binds to CEA-Rs on Kupffer cells and up-regulates the production of cytokines such as TNF-α, which in turn facilitate the increased retention and survival of metastatic tumor cells within the liver [18]. They also greatly enhance the ability of metastasizing

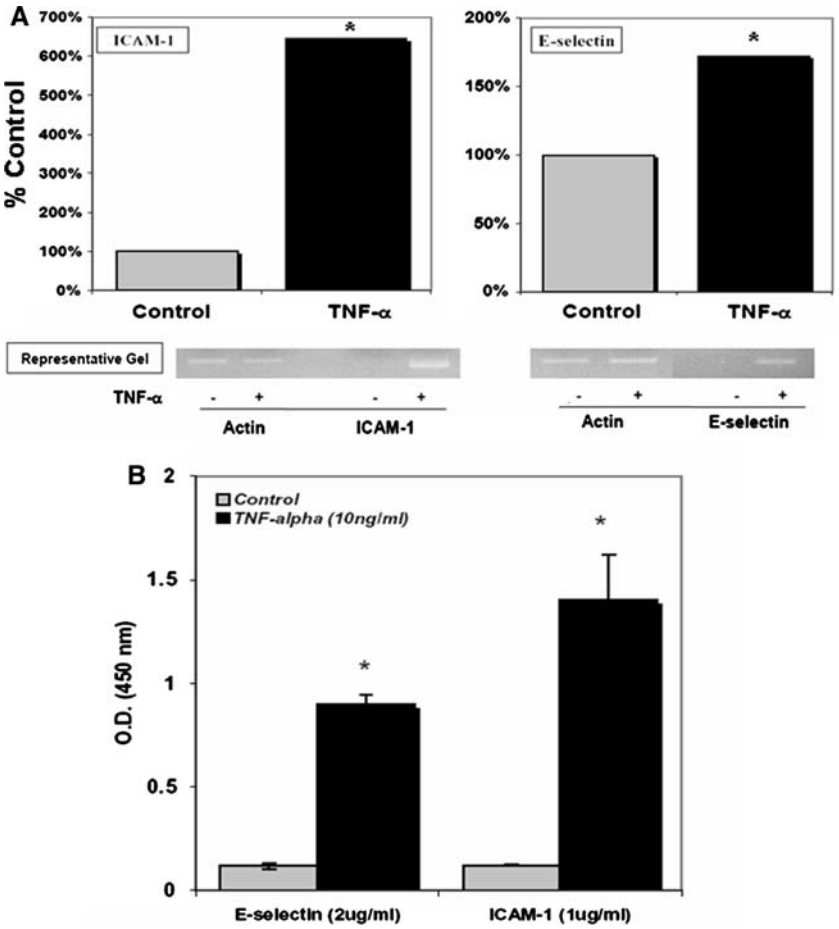

Fig. 4 Expression of E-selectin and ICAM-1 mRNA and protein in TNF-α stimulated human umbilical vein endothelial cells. Recombinant TNF-α (10 ng/ml) significantly increased the (**a**) mRNA and (**b**) cell-surface expression of the adhesion molecules E-Selectin and ICAM-1. Results shown as means ± SEM. * $P < 0.05$ compared to controls. *ICAM-1* intercellular adhesion molecule-1, *TNF-α* tumor necrosis factor alpha

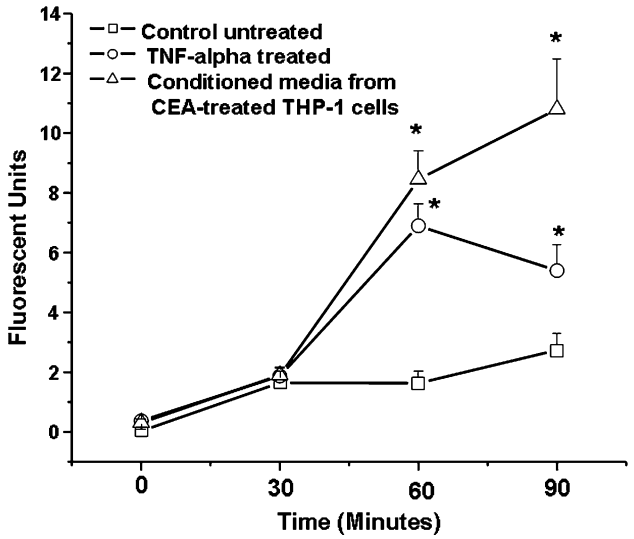

Fig. 5 Attachment of HT-29 human colon cancer cells to activated human umbilical vein endothelial cells. The attachment of fluorescently labeled HT-29 cells to HUVECs was significantly increased following the pre-incubation of HUVECs with either TNF-α or conditioned media from differentiated THP-1 cells treated with CEA (2 μg/ml). Results shown as means ± SEM. * $P < 0.05$ compared to control $n = 3$ in triplicate. *HUVECs* human umbilical vein endothelial cells, *TNF-α* tumor necrosis factor alpha

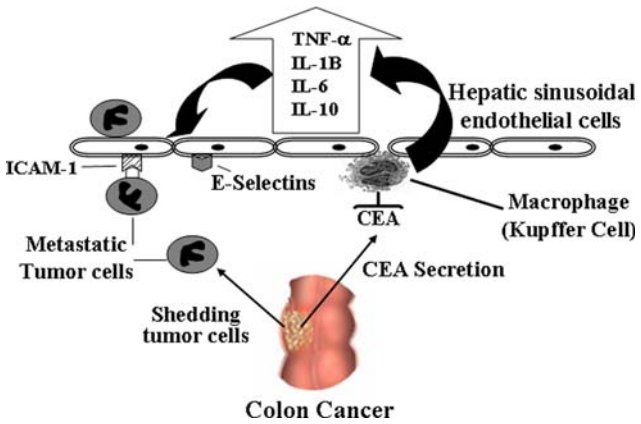

Fig. 6 A working model depicting the activation of Kupffer cells by CEA. CEA, secreted from colorectal cancer cells, binds to CEA Receptors on Kupffer cells and induces the release of proinflammatory cytokines, which in turn up-regulate the cell-surface expression of adhesion molecules on hepatic sinusoidal endothelial cells. These adhesion molecules facilitate the attachment of circulating tumor cells to the endothelium, which exacerbates the metastatic process. *CEA* carcinoembryonic antigen, *CEA-R* CEA receptor, *TNF-α* tumor necrosis factor alpha, IL (interleukin), *ICAM-1* intercellular adhesion molecule-1

colon cancer cells to attach and translocate into the liver by inducing adjacent hepatic sinusoidal vascular endothelial cells to up-regulate their cell surface expression of adhesion molecules, such as ICAM-1 and E-selectin. Clinical confirmation of tumor spread to the liver by increased serum CEA levels is a negative prognostic sign, as only a small percentage of patients with liver metastases are candidates for potentially curative surgical resection. Thus, along with early metastatic tumor detection, a better understanding of the metastatic process will improve the overall outcome for the metastatic cancer patient.

In conclusion, this study establishes an in vitro system utilizing the D-THP human macrophage cell line expressing functional CEA-Rs to study activation and signaling mechanisms of CEA that facilitate tumor cell attachment to activated endothelial cells; a key step in the metastatic process. While D-THP cells do not recapitulate Kupffer cells, this in vitro system does offer a model that can be very useful for the investigation of CEA-mediated signaling processes that mediate metastasis to the liver. Utilization of this in vitro system may lead to a more complete understanding of the expression and function of the CEA-R and facilitate the design of anti-CEA-R therapeutic modalities that may significantly diminish the metastatic potential of CEA overexpressing colorectal tumors.

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