COMPARISON OF IMMUNOLIPOSOME TARGETING TO THREE DIFFERENT RECEPTORS EXPRESSED BY HUMAN OVARIAN CARCINOMA (OVCAR-3) CELLS
COMPARISON OF IMMUNOLIPOSOME TARGETING TO THREE DIFFERENT RECEPTORS EXPRESSED BY HUMAN OVARIAN CARCINOMA (OVCAR-3) CELLS

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SUMMARY

Immunoliposomes were targeted to three different cell-surface receptors: intercellular adhesion molecule-1 (ICAM-1), epithelial glycoprotein-2 (EGP-2), and epidermal growth factor receptor (EGFR) expressed on OVCAR-3 ovarian carcinoma cells. Binding and internalization of these immunoliposomes was analyzed qualitatively by flow cytometry and confocal laser scanning microscopy to determine which cell-surface receptor is most suitable in intracellular delivery of immunoliposomal drugs to ovarian carcinoma cells. Under the tested conditions, the EGFR turned out to be the best candidate to obtain receptor-mediated endocytosis of a large amount of immunoliposomes.
The research described in this thesis aims at delivering therapeutic compounds encapsulated in antibody-directed liposomes (immunoliposomes; IL) into the cytoplasm of tumor cells, in particular of ovarian carcinoma cells (OVCAR-3) cells. This is attempted by exploiting the route of receptor-mediated endocytosis followed by endosomal escape of immunoliposome-entrapped drug molecules, induced by pH-dependent fusogenic peptides or proteins incorporated in the liposomal carrier. This 'Trojan horse' approach of delivery requires binding and internalization of immunoliposomes as a first step in the cytosolic delivery of entrapped drugs.

Both the occurrence of internalization and lack of internalization of immunoliposomes specifically bound to target cell surface receptors have been reported. The occurrence of receptor-mediated endocytosis of receptor-bound immunoliposomes is determined by the type of receptor, provided that immunoliposomes are small enough (<150 nm). Therefore, the choice of the target receptor for intracellular delivery of immunoliposomal drugs is of major importance. However, not all monoclonal antibodies recognizing the same receptor mediate internalization of the receptor/mAb complex. It was demonstrated that three different mAbs recognizing different epitopes on the same receptor (folate-binding protein) on ovarian carcinoma cells were processed by the cells in different ways. Two out of three mAbs were internalized, whereas the other remained located on the cell surface. Thus, not all mAbs directed against receptors with internalizing capacity will be able to trigger internalization as these mAb recognize different epitopes on the same receptor. In addition, the internalizing capacity of cell-surface receptors is also dependent on the type of cell that expresses the cell-surface receptor. For instance, immunoliposomes targeted to the HER-2 antigen were reported to be efficiently internalized by human breast cancer cells, less efficiently by MKN-7 human gastric or SKOV-3 ovarian carcinoma cells, and not at all by N-87 human gastric carcinoma cells. Consequently, internalization of antibody-directed liposomes cannot be predicted a priori.

For targeting immunoliposomes to ovarian carcinoma cells, mAbs directed against several receptors have been used. In our group Fab’ fragments of mAb OV-TL3 directed against the tumor-associated antigen OA3, which is present on more than 90% of all human ovarian carcinomas and mAb 323/A3 directed against the epithelial glycoprotein-2 (EGP-2) were conjugated to the surface of doxorubicin-laden liposomes. For both OV-TL3 immunoliposomes and 323/A3 immunoliposomes specific binding to OVCAR-3 was demonstrated in vitro and in vivo. Binding of OV-TL3 immunoliposomes to OVCAR-3 cells residing in the peritoneal cavity of nude mice after i.p. injection was high: after 5 hrs incubation >80% of immunoliposomes recovered from the peritoneal cavity was found to be associated with tumor cells. In addition, electron microscopy analysis demonstrated that OV-TL3 immunoliposomes were bound to the surface of OVCAR-3 cells that were isolated from the peritoneal cavity of athymic mice. Internalization of cell-bound immunoliposomes was not detected after this 5 hrs incubation.
Internalization of 323/A3-IL by OVCAR-3 cells has not been investigated so far. It has been shown in the immunotoxin literature that targeting to the EGP-2 receptor can lead to internalization: 25% of cell bound immunotoxins consisting of doxorubicin conjugated to mAb MOC31 (α-EGP-2), were internalized by small lung cancer cells after 5 hrs. Besides the above mentioned target receptors, other receptors may be used to target immunoliposomes to OVCAR-3 cells. In a study from our group immunoliposomes were targeted to intercellular adhesion molecule-1 (ICAM-1) expressed on human bronchial epithelial cells. It was demonstrated that the immunoliposomes were capable to specifically bind to these cells; in addition, they were efficiently internalized (see chapter 2 and ). As ICAM-1 expression is not only up-regulated at sites of inflammation but is also involved in the metastatic spread of many types of tumor cells, this receptor may also be used for targeting immunoliposomes to OVCAR-3 cells. The epidermal growth factor receptor (EGFR), has been found to be over-expressed on many carcinoma cells and is often used as target for drug delivery to carcinoma cells.

In this study we have targeted immunoliposomes to the cell-surface receptors ICAM-1, EGP-2 and EGFR expressed on OVCAR-3 cells. Binding and internalization by OVCAR-3 cells was monitored qualitatively by flow cytometry and confocal laser scanning microscopy to determine which antibody is most suitable to achieve specific internalization of immunoliposomes by OVCAR-3 cells. The results show that the EGFR is the most suitable candidate to deliver immunoliposomes into the endocytic pathway of OVCAR-3 cells.
MATERIALS & METHODS

MATERIALS

Cholesterol (CHOL), N-succinimidyl-5-acetylthioacetate (SATA), N-ethylmaleimide, bovine serum albumin (fraction V), fluorescein isothiocyanate (FITC)-labeled goat anti-mouse IgG (Fab’-specific) and R-phycoerythrin (PE)-labeled goat anti-mouse IgG were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). Chloroform and methanol were obtained from Merck (Darmstadt, Germany), and N,N-dimethylformamide (DMF) and hydroxylamine hydrochloride from Janssen Chimica (Geel, Belgium). Partially hydrogenated egg-phosphatidylcholine with an iodine value of 40 (PHEPC; Asahi Chemical Industry Co., Ibarakiken, Japan) was prepared as described previously. N-[4-(p-maleimidophenyl) butyryl]phosphatidylethanolamine (MPB-PE) and lissamine rhodamine B-labeled glycerophosphoethanolamine (Rho-PE) were purchased at Avanti Polar Lipids Inc. (Alabaster, AL, USA). Anti-ICAM-1 mAb (IgG1), clone F10.2 was a gift from Dr. Henricks (department of Pharmacology and Pathophysiology, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, The Netherlands). Murine mAb 425 of isotype IgG2b (EMD55900) (Merck KGaA, Bernstadt, Germany) directed against the human epidermal growth factor receptor (EGFR) was kindly donated by Dr. G.A. van Dongen (Department of Otolaryngology/Head and Neck Surgery, University Hospital Vrije Universiteit, Amsterdam, The Netherlands). Monoclonal antibody 323/A3, directed against the epithelial glycoprotein-2 (EGP-2) was kindly donated by Centocor Europe BV (Leiden, The Netherlands). Formaldehyde was obtained from Janssen Chimica (Geel, Belgium). 1,1’-dioctadecyl-3,3,3’,3’-tetramethylindodicarbocyanine perchlorate (DiD) was purchased from Molecular Probes (Leiden, The Netherlands).

CELL CULTURE

The human ovarian carcinoma cell line NIH:OVCAR-3 was originally obtained from Dr. Hamilton (National Cancer Institute, Bethesda, MD). OVCAR-3 cells were cultured in DMEM containing 3.7 g/l sodium bicarbonate, 4.5 g/l L-glucose (Gibco, Grand Island, NY, USA) and supplemented with 2 mM L-glutamine, 10 mM HEPES, 10% (v/v) heat inactivated fetal calf serum (FCS), penicillin (100 IU/ml), streptomycin (100 µg/ml), and amphotericin B (0.25 µg/ml; all purchased at Gibco). Cells were maintained at 37°C in humidified air with 5% CO₂.
**PREPARATION AND CHARACTERIZATION OF IMMUNOLIPOSOMES**

Immunoliposomes were prepared by conjugating mAb 425 (IgG2b), 323/A3 (IgG1), and F10.2 (IgG1) to the membrane of liposomes according to the method described previously with some minor modifications (see chapter 2). In short, liposomes were made by hydrating a lipid film composed of PHEPC, CHOL and MPB-PE at a molar ratio of 1.925:1:0.075, respectively in 5 mM HEPES, 150 mM NaCl, 1 mM EDTA, pH 7.4 (HBS/EDTA). When indicated either NBD-PE or Rho-PE were included in the liposomes as a fluorescent marker at a molar amount of 0.1% relative to the total amount of lipid. Subsequently, liposomes were sized by extrusion through 0.1 μm pore filters. Freshly thiolated mAbs (for details see chapter 2) at a final concentration of 450 µg/ml were added to the liposomes (40 mM) and incubated for 2 hrs at room temperature under constant rotation. The coupling reaction was terminated by adding 50 µl of freshly prepared 8 mM N-ethylmaleimide in HEPES buffer (10 mM HEPES; 1mM EDTA; 135 mM NaCl pH 7.4). Liposomes were separated from unconjugated mAbs by ultracentrifugation (4 runs of 30 min at 60,000xg) and stored at 4°C. Mean particle size of (immuno)liposomes was determined by dynamic light scattering as described before. Phospholipid concentration was determined by the colorimetric method of Fiske and Subbarow and the amount of conjugated IgG on the liposomal surface was determined with the Bio-Rad DC protein assay kit (Bio-Rad Laboratories, Hercules, CA, USA) with mouse IgG as standard.

**FLOW CYTOMETRY ANALYSIS OF IMMUNOLIPOSOME BINDING TO OVCAR-3 CELLS**

Immunoliposomes and control liposomes (without conjugated mAb) were added to 2.5x10^5 OVCAR-3 cells and incubated for 1 h at the indicated temperature. Cells were washed 2 times with 1 ml of immunofluorescence (IF) buffer (1% (w/v) BSA in PBS pH 7.4). Hereafter, cells that had been incubated with fluorescently labeled immunoliposomes (i.e. immunoliposomes containing Rho-PE as a lipidic fluorescent marker) were resuspended in 500 µl of IF-buffer and analyzed by flow cytometry. To cells that were incubated with unlabeled (immuno)liposomes, 100 µl of appropriately diluted FITC-labeled goat anti-mouse IgG were added and incubated for 1 h on ice. After two additional wash steps with IF-buffer, these cells were resuspended into 500 µl IF-buffer and analysed for cell-associated fluorescence by flow cytometry. Quantification of the degree of cellular internalization of cell-bound liposomes was performed as previously described by us and another group (see also chapter 2).
CONFOCAL LASER SCANNING MICROSCOPY (CLSM) ANALYSIS
OF CELLULAR UPTAKE OF IMMUNOLIPOSOMES

For CLSM analysis, OVCAR-3 cells, either in suspension or adhered to chamber slides (Nalge Nunc International Corp., Naperville, IL, USA), were incubated with Rho-PE-labeled (immuno)liposomes for 1 h after which cells were washed twice with 1 ml of PBS and subsequently fixed with 4% (v/v) of formaldehyde in PBS for 20 min at room temperature. After fixation, cells were washed twice with PBS. Suspension cells were mounted on object slides and overlaid with cover slides that were sealed with nail polish. Cells were analyzed on a Leica TCS-SP confocal laser-scanning microscope equipped with a 488 nm Argon, 568 nm Krypton and 647 nm HeNe laser.

Figure 1 – Expression pattern of different cell-surface receptors on the surface of OVCAR-3 cells determined by indirect immunofluorescence staining and flow cytometry. Shown are OVCAR-3 cells incubated with FITC-labeled goat anti-mouse secondary antibody (a), with mAb 425 (αEGFR) and FITC-labeled secondary antibody (b); with mAb F10.2 (αICAM-1) and FITC-labeled secondary antibody (c), and with mAb 323/A3 and FITC-labeled secondary antibody (d). Histograms show the mean fluorescent intensity of 4000 cells.
RESULTS AND DISCUSSION

In this study, immunoliposomes were targeted to three different cell surface receptors on OVCAR-3 cells to explore which receptor is most efficient in delivering immunoliposomes into the endosomal pathway of the cell as the first step in a strategy for cytosolic drug delivery. Based on previous experience with immunoliposome targeting, three different surface receptors were chosen: the intercellular adhesion-molecule-1 (ICAM-1), the epithelial glycoprotein-2 (EGP-2) and the epidermal growth factor receptor (EGFR). The expression pattern of these cell-surface receptors on OVCAR-3 cells was determined by flow cytometry with indirect immunofluorescence using monoclonal antibodies directed against EGP-2 (mAb 323/A3), EGFR (mAb 425) and ICAM-1 (mAb F10.2). The expression levels of the different cell surface receptors are shown in Figure 1. The expression level of EGP-2 was 10 times higher than the expression levels of ICAM-1 and EGFR, which showed comparable expression levels. For liposome targeting, the 3 mAbs were chemically modified with SADA to allow covalent conjugation to the surface of liposomes. The average size of liposomes and the amount of conjugated IgG are given in Table 1.

IMMUNOLIPOSOME BINDING TO OVCAR-3 CELLS

Binding of fluorescently labeled immunoliposomes to OVCAR-3 cells at 4°C is shown in Figure 2. Both 323/A3-IL and 425-IL showed concentration-dependent binding to OVCAR-3 cells. Binding of 323/A3-IL was higher than binding of 425-IL at concentrations >1 μmol

<table>
<thead>
<tr>
<th>Type of Liposomes</th>
<th>Average particle size (nm) / Polydispersity Index</th>
<th>Amount of conjugated mAb (μg/μmol PL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Liposomes</td>
<td>133/0.08</td>
<td>—</td>
</tr>
<tr>
<td>425-Immunoliposomes (α-EGFR)</td>
<td>128/0.09</td>
<td>10</td>
</tr>
<tr>
<td>323/A3-Immunoliposomes (α-EGP-2)</td>
<td>155/0.13</td>
<td>18</td>
</tr>
<tr>
<td>F10.2-Immunoliposomes (α-ICAM1)</td>
<td>131/0.09</td>
<td>14</td>
</tr>
</tbody>
</table>
Binding of control liposomes (i.e. liposomes without conjugated antibody) was negligible at the concentration range tested. Surprisingly, immunoliposomes targeted to ICAM-1 did not show any binding to OVCAR-3 cells despite the fact that ICAM-1 cell-surface expression on these cells was detected. It is not clear which factors underlie this lack of cell binding. One potential reason may be that modification of mAb F10.2 with SATA necessary to conjugate the antibody to maleimide-derivatized phospholipids in the liposome bilayer has resulted in loss of binding activity of this mAb. In chapter 2, however, it was demonstrated that modification of F10.2 with SATA was possible with preservation of binding activity. Yet, F10.2 used in chapter 2 was modified at a lower SATA to antibody ratio (8:1) compared to SATA modification of mAb F10.2 used in this study (30:1). Due to the inability of F10.2 IL to bind to OVCAR-3 cells, these immunoliposomes were excluded from further experiments.

Analysis of immunoliposome binding by confocal laser scanning microscopy (CLSM) confirmed that both 323/A3-IL and 425-IL are able to bind to a monolayer of OVCAR-3 cells adhered to polystyrene chamber slides as indicated by the presence of rhodamine-fluorescence on the surface of the cells (Figure 3). Fluorescent staining of OVCAR-3 cells incubated with 425-IL is relatively weak compared to cells incubated with 323/A3-IL, indicating a lower degree of cell binding of

![Figure 2 – Binding of immunoliposomes to OVCAR-3 cells at 4°C. OVCAR-3 cells were incubated with liposomes labeled with the lipophilic fluorescent probe Rho-PE for 1 h at 4°C. After removal of unbound liposomes cells were analyzed for cell-associated rhodamine fluorescence by flow cytometry. Each point represents the mean fluorescence intensity of 4000 cells analyzed (n=3). 323/A3 IL: mAb 323/A3-directed immunoliposomes; F10.2 IL: mAb F10.2-directed immunoliposomes; 425-IL: mAb 425-directed immunoliposomes; CL: control liposomes without conjugated antibodies, but with the same lipid composition as the immunoliposomes. Error bars indicate SEM (n=3).]
Figure 3 – Confocal laser scanning microscopy (CLSM) analysis of immunoliposome binding to OVCAR-3 cells at 4ºC. A monolayer of OVCAR-3 cells adhered to chamber slides was incubated with Rho-PE-labeled 323/A3-IL (Panel A) or 425-IL (Panel B) for 60 min at 4ºC (500 nmol PL/ml). After removal of unbound liposomes, cells were fixed in 4% formaldehyde solution in PBS and analyzed by CLSM and light microscopy (LM). For clarity, cell-associated rhodamine fluorescence is indicated in greyscale (white indicates no fluorescence, black indicates highest fluorescence). Pictures obtained with CLSM and LM have been superimposed to show the localization of fluorescence inside cells. Scale bar represents 25 µm.
425-IL at 4°C. The observed cell-associated fluorescence is primarily located at the plasma membrane of OVCAR-3 cells with no detectable spots of fluorescence located intracellularly. Binding of immunoliposomes to both OVCAR-3 cells and A-2780 cells (ovarian carcinoma cells lacking expression of EGP-2) was also evaluated at 37°C both with flow cytometry and CLSM. For flow cytometry analysis, 323/A3 immunoliposomes and 425-immunoliposomes were labeled with the fluorescent probe DiD. Figure 4 shows that the degree of binding of 323/A3-IL to OVCAR-3 cells is again higher compared to that of 425-IL. In addition, 323/A3-IL binding was restricted to OVCAR-3 cells, whereas 425-IL also bound to A2780 cells, although less than binding to OVCAR-3 cells. CLSM analysis with Rho-PE-labeled immunoliposomes demonstrates that cell-associated fluorescence is more intense at 37°C compared to binding at 4°C (Figure 5), indicating that the degree of binding is higher at 37°C. Interestingly, the distribution of cell-bound 323/A3-IL is primarily confined to cells located at the edges of the monolayer, whereas 425-IL binding is more equally distributed over the cells present in the monolayer. Basolateral expression of EGP-2 epithelial cells has been demonstrated in a study comparing the expression patterns of EGP-2 in 84 different human colorectal adenomas and adeno-carcinomas as well as healthy tissue. Although the complex organization of epithelial cells in vivo cannot be compared with the in vitro situation it might well be that EGP-2 expression on adherent OVCAR-3 cells growing as a monolayer is restricted to the basolateral side of the cells, which may explain the

**Figure 4** – Flow cytometry analysis of immunoliposome binding to OVCAR-3 and A2780 cells as a function of liposome concentration. Cells were incubated with increasing concentrations of DiD-labeled immunoliposomes or control liposomes for 60 min at 37°C. Cells were analyzed by flow cytometry after removal of unbound liposomes. Each point represents the average of the mean fluorescence intensity of 4000 cells. Error bars indicate SEM (n=3).
Figure 5 – CLSM analysis of immunoliposome binding to OVCAR-3 cells at 37°C. A monolayer of OVCAR-3 cells adhered to chamber slides were incubated with rhodamine-labeled 323/A3-IL (Panel A) or 425-IL (Panel B) for 60 min at 37°C (500 nmol PL/ml). After removal of unbound liposomes, cells were fixed in 4% formaldehyde solution in PBS and analyzed by CLSM and light microscopy (LM). For clarity, cell-associated rhodamine fluorescence is indicated in greyscale (white indicates no fluorescence, black indicates highest fluorescence). Pictures obtained with CLSM and LM have been superimposed to show the localization of fluorescence inside cells. Scale bar represents 25 µm.
Figure 6 – CLSM analysis of immunoliposome binding to OVCAR-3 cells in suspension. Panel A: OVCAR-3 cells incubated with 500 µmol/ml PL of 425-IL for 1 h at 37°C. Panel B: same as panel A but in the presence of 1 mg/ml free mAb 425; Panel C: same as panel A but in the presence of 1 mg/ml of free mAb 323/A3. Scale bars represent 25 µm.
lower degree of cell binding. Besides cell membrane-associated fluorescence also spots of fluorescence located inside cells can be discerned when the cells were incubated with 425-IL. The frequency and intensity of intracellular fluorescent spots is much less when cells were incubated with 323/A3-IL, indicating that 425-IL are more efficiently internalized by OVCAR-3 cells than 323/A3-IL.

Binding of 425-IL to OVCAR-3 cells could be blocked by adding high concentrations (1 mg/ml) of free mAb 425 to the cells prior to the addition of the immunoliposomes. Pre-incubation with the same concentration of mAb 323/A3 did not have an effect on the degree of 425-IL binding to OVCAR-3 cells (Figure 6). These results strongly indicate that 425-IL binding to OVCAR-3 cells is exclusively mediated by the conjugated monoclonal antibodies present on the surface of 425-IL.

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**Figure 7** – Determination of the amount of immunoliposomes bound to the surface of OVCAR-3 cells. OVCAR-3 cells, both vital and fixed with 4% formaldehyde, were incubated with immunoliposomes (500 µmol PL/ml) for 1 h at 4°C. After removal of unbound liposomes, cells were further incubated in culture medium at 37°C for indicated time periods. Hereafter, immunoliposomes still present on the cell surface were stained with a FITC-labeled goat anti-mouse antibody and analyzed by flow cytometry. The amount of immunoliposomes present on the cell surface is expressed relative to the initial amount of cell-bound immunoliposomes (i.e. at t=0 min, directly after incubation at 4°C). Error bars indicate SEM (n=3).
Figure 8 – CLSM close-up analysis of immunoliposome internalization into OVCAR-3 cells. OVCAR-3 cells were incubated with 500 µmol PL/ml of immunoliposomes for 2 hrs at 37°C. Unbound immunoliposomes were removed prior to fixation of cells with 4% formaldehyde. Panel A: OVCAR-3 cells grown in suspension and incubated with 323/A3 IL. Panel B: OVCAR-3 cells grown in suspension and incubated with 425-IL. Panel C: OVCAR-3 cells grown as a monolayer and incubated with 425-IL. Scale bars represent 10 µm.
Internalization of cell-bound immunoliposomes was also confirmed by indirect immunofluorescence analysis as described previously. Immunoliposomes were incubated with OVCAR-3 cells at 4°C for 1 h. After removal of unbound immunoliposomes, cells were incubated at 37°C for the indicated time period. Then the immunoliposomes still attached to the cell-surface of OVCAR-3 cells were labeled with FITC-conjugated antibodies directed against the liposome-conjugated mAbs. The results of this experiment are shown in Figure 7. It can be seen that the amount of immunoliposomes located on the surface of OVCAR-3 cells decreased within time. After 2 hrs, the amount of 323/A3-IL initially bound to OVCAR-3 cells was decreased with approx. 25% and the amount of 425-IL with approx. 50%. Control experiments using cells fixed with formaldehyde prior to immunoliposome binding show a marginal reduction in immunoliposome binding (approx. 10%). As fixed cells do not internalize particles, differences in reduction of immunoliposomes bound to the surface of OVCAR-3 cells between fixed and non-fixed cells can be ascribed to an active process of the cell, which strongly suggests that both 323/A3-IL and 425-IL are internalized by OVCAR-3 cells.

To ascertain the occurrence of internalization, close-ups of OVCAR-3 cells incubated with either 323/A3-IL or 425-IL were made by confocal laser scanning microscopy to check for intracellularly localized immunoliposomes (Figure 8). 323/A3-IL incubated with OVCAR-3 cells at 37°C are primarily located at or near the cell membrane of OVCAR-3 cells as illustrated by a rim of fluorescence around OVCAR-3 cells. Only minor amounts of internalized spots of fluorescence can be observed (Figure 8A). In contrast, 425-IL incubation yield many fluorescent spots inside OVCAR-3 cells besides fluorescence at the cell surface (Figure 8B). Internalization of 425-IL by OVCAR-3 cells is more pronounced when adherent OVCAR-3 cells are used as compared to OVCAR-3 cells in suspension (Figure 8C).

Taking these results together it can be concluded that both EGP-2 and EGFR can be used as receptors to target immunoliposomes into the endosomal pathway of OVCAR-3 cells. Although the expression level of EGP-2 is much higher than the expression level of EGFR, the capacity of this receptor to internalize immunoliposomes is much lower (~15% of cell-bound IL after 2 hrs) as compared to the EGFR (~40% after 2 hrs). This finding together with the poor accessibility of EGP-2 on OVCAR-3 cells observed in vitro suggest that the EGF-receptor is the most suitable candidate of the 3 tested for achieving receptor-mediated endocytosis of immunoliposomes and their entrapped drugs by OVCAR-3 cells.
REFERENCE LIST


