Journal of Cellular Biochemistry 71:149–157 (1998)

ARTICLES

Expression of α_2-Macroglobulin Receptor-Associated Protein in Normal Human Epidermal Melanocytes and Human Melanoma Cell Lines

Yonghe Li,[1,2] **Nick Wood,**[1]* **David Yellowlees,**[2] **and Peter K. Donnelly**[1]

[1]Department of Surgery, North Queensland Clinical School, University of Queensland, Townsville, 4810 Australia
[2]Department of Biochemistry and Molecular Biology, James Cook University, North Queensland, Townsville, 4811 Australia

Abstract α_2-Macroglobulin receptor/low-density lipoprotein receptor-related protein is a multifunctional cell surface receptor known to bind and internalize a large number of ligands. α_2-Macroglobulin receptor-associated protein acts as an intracellular "chaperone" for this receptor, and it has been shown to inhibit binding of all its known ligands. In this paper, we characterize the expression of the receptor-associated protein in both normal human epidermal melanocytes and in six different human melanoma cell lines, by the use of flow cytometry and Western blotting analysis. We show that all the melanoma cell lines and the normal melanocytes express the receptor-associated protein at similar levels, with most located intracellularly. No receptor-associated protein was detected at the cell surface in the melanocytes or in three of the cell lines. However, in two of the melanoma cell lines, large amounts of receptor-associated protein were found on the cell surface, these having the largest amounts of it reported to date; in a further melanoma cell line, there was a small amount at the cell surface. We have also shown that the melanocytes and all the melanoma cell lines express the receptor itself at a wide range of levels, the highest levels of both the cell surface receptor and the cell surface receptor-associated protein being found in one particular melanoma cell line. By growing the cell lines under controlled conditions, we have demonstrated that, although the total cellular content of the receptor is markedly increased at high cell culture density, this treatment has no effect on the level of expression of the receptor-associated protein. J. Cell. Biochem. 71:149–157, 1998.

Key words: RAP; α_2MR/LRP; melanocytes; melanoma; cell culture density; flow cytometry

α_2-Macroglobulin receptor/low-density lipoprotein receptor-related protein (α_2MR/LRP) is a member of the low-density lipoprotein receptor (LDLR) family which, as well as LDLR itself, includes α_2MR/LRP, glycoprotein 330 (gp330), the very-low-density lipoprotein receptor (VLDLR) [Krieger and Herz, 1994; Moestrup, 1994], apolipoprotein E receptor-2 (apoER2) [Kim et al., 1996], and LR8B, which is closely related to apoER2 [Novak et al., 1996]. α_2MR/LRP is synthesized as a 600-kDa single-chain precursor that undergoes post-translational proteolytic processing to form a M_r 515-kDa heavy chain and a M_r 85-kDa light chain [Herz et al., 1988, 1990]. The light chain includes a transmembrane domain [Herz et al., 1990], and it is bound to the heavy chain by nonconvalent interactions. The heavy chain has binding sites for a number of structurally and functionally diverse ligands, including proteases, protease inhibitors, and lipoprotein particles [Krieger and Herz, 1994; Moestrup, 1994].

α_2-Macroglobulin receptor-associated protein (RAP) is a 39-kDa polypeptide that is co-purified with α_2MR/LRP [Jensen et al., 1989; Ashcom et al., 1990; Moestrup et al., 1991] and with gp330 [Orlando et al., 1992; Kounnas et al., 1992]. RAP binds with high affinity to α_2MR/LRP [Herz et al., 1991]; it also binds to gp330 [Christensen et al., 1992; Kounnas et al., 1992], to VLDLR [Simonsen et al., 1994; Battay et al., 1994] and to LDLR, but with lower affinity to the latter [Mokuno et al., 1994; Medh et al., 1995]. Studies have demonstrated that RAP is

Contract grant sponsor: University of Queensland.

*Correspondence to: Dr. Nick Wood, Department of Surgery, North Queensland Clinical School, University of Queensland, Townsville, 4810 Australia.

Received 19 March 1998; Accepted 6 May 1998

able to inhibit the binding of all known ligands of the LDLR family members [Krieger and Herz, 1994; Moestrup, 1994].

The expression and distribution of RAP on human tissues has not been extensively studied. RAP is known to co-purify with α_2MR/LRP from human liver [Moestrup et al., 1991] and placenta [Jensen et al., 1989; Ashcom et al., 1990] and with gp330 from rat kidney [Orlando et al., 1992; Kounnas et al., 1992]. In the rat, RAP is found in all cells that express α_2MR/LRP or gp330 [Zheng et al., 1994].

Although the presence of RAP at the cell surface remains controversial, many studies indicate that RAP resides predominantly in the endoplasmic reticulum (ER) and in the Golgi complex [Orlando et al., 1992; Lundstorm et al., 1993; Bu et al., 1994] and it serves as a chaperone for α_2MR/LRP [Bu et al., 1995; Willnow et al., 1995, 1996; Bu and Rennke, 1996; Obermoeller et al., 1997]. It has been shown that RAP functions as a regulator of α_2MR/LRP activity by transiently interacting with it and maintaining it in an inactive, non-ligand-binding state while it is in the ER. When RAP dissociates from α_2MR/LRP in response to the lower pH within the Golgi, the α_2MR/LRP becomes active as it is transported to the cell surface [Bu et al., 1995; Willnow et al., 1996]. Other studies suggest that RAP is also required for the process of folding the α_2MR/LRP protein to assume its tertiary configuration by preventing the formation of intermolecular disulfide bonds [Bu and Rennke, 1996]. The importance of RAP in the maturation and trafficking of α_2MR/LRP has been demonstrated by gene-knockout studies [Willnow et al., 1995], which have shown that cells lacking RAP suffer a 75% reduction of functional α_2MR/LRP.

Little is known about RAP expression in human tumor cell lines or in human tumor tissues. Co-location of RAP and α_2MR/LRP has been described for glioblastoma cells [Bu et al., 1994]. It has been reported that RAP and α_2MR/LRP are co-located in human melanoma cell lines, and that RAP mRNA and protein are expressed at corresponding levels intracellularly, with no cell surface expression in vitro. This study has also shown that they are coordinately expressed in a decreasing fashion in cutaneous melanocytic tumor progression in vivo [de Vries et al., 1996]. In human prostate carcinoma, the levels of RAP mRNA remain constant, whereas the α_2MR/LRP mRNA increases significantly in high Gleason score carcinoma and in metastatic lesions [McGarvey et al., 1996]. In human hepatoma cell line HepG2, where dexamethasone up-regulates α_2MR/LRP, there is no effect on RAP expression [Kancha et al., 1996].

We have previously demonstrated that human melanoma cell lines express cell surface α_2MR/LRP [Li et al., 1997a]. In this report, we characterize the expression of RAP in both human melanoma cell lines and normal human epidermal melanocytes.

METHODS

Cell Culture

The human melanoma cell lines MM96L, MM138, MM253c1, MM370, MM418c1, and A2058, and the normal human neonatal foreskin fibroblasts cell line NFF were kindly supplied by Dr. P.G. Parsons (Queensland Institute of Medical Research, Brisbane, Queensland, Australia). NFF was used as a positive control for RAP and α_2MR/LRP. All the tumor cell lines and NFF were cultured in RPMI-1640 (Sigma Chemical Co., St. Louis, MO) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (CSL-Novo, North Rocks, NSW, Australia), 6 mM L-glutamine (Sigma), 20 mM HEPES (Sigma), 100 U/ml penicillin G, and 100 µg/ml streptomycin sulfate (Sigma) at 37°C in an atmosphere of 5% CO_2. Cells were passaged routinely every 3–5 days at about 80–90% confluency. The cells were briefly treated with 0.05% trypsin/0.02% EDTA (Sigma) in calcium and magnesium–free Hank's balanced salt solution (HBSS).

The normal human epidermal melanocytes (NHEM) were obtained from Clonetics (San Diego, CA). NHEM were cultured as recommended by the supplier in melanocyte growth medium (MGM) containing 15 µg/ml bovine pituitary extract, 1 ng/ml human fibroblast growth factor-B, 5 µg/ml bovine insulin, 10 ng/ml phorbol 12-myristate 13-acetate (PMA), 0.5 µg/ml hydrocortisone, 50 µg/ml gentamicin sulfate, and 0.5% FBS. The cells were incubated at 37°C in an atmosphere of 5% CO_2, changing the medium every other day, and subculturing the cells when they reached 70–80% confluency.

Antibodies

The mouse monoclonal IgG1 antibody to human RAP (7F1) was purchased from Research

Diagnostics (Flanders, NJ). 7F1 reacts with the 39-kDa RAP protein and has been used in ligand binding assays, Western blotting, and immunohistochemistry [Kounnas et al., 1992]. The rabbit polyclonal antiserum against RAP was generously provided by Dr. Alan Schwartz (Department of Paediatrics, Washington University School of Medicine, St. Louis, MO); it has previously been used in immunohistochemistry and flow cytometry [Bu et al., 1994; de Vries et al., 1996].

The mouse monoclonal IgG1 antibody to human α_2MR/LRP (8G1) was kindly provided by Dr. Dudley Strickland (American Red Cross, Rockville, MD). This antibody is specific for the 515-kDa heavy chain of α_2MR/LRP; it has been well characterized for use in immunohistochemistry, Western blotting, and flow cytometry [Strickland et al., 1990; Coukos et al., 1994; Lopes et al., 1994; Li et al., 1997a].

RAP Determination by Flow Cytometry

To determine total cellular RAP expression, the target cells must be fixed and made permeable to antibodies. For this, suspensions of $\sim 1 \times 10^7$ cells in 50 µl of phosphate-buffered saline (PBS)/FBS (PBS containing 1.5% heat-inactivated FBS and 0.1% sodium azide) were treated with fluorescence-activated cell sorting (FACS) permeabilizing solution (Permeafix; Becton Dickinson, San Jose, CA) adding 500 µl to each aliquot with gentle mixing and incubating at room temperature for 8 min in the dark. The cells were washed by centrifugation at 200*g* for 5 min in 2 ml PBS/FBS/1% saponin (Sigma) and incubated with 50 µl 7F1. Further samples were incubated with the same amount of a monoclonal IgG1 isotype control of unrelated specificity. The cells were then washed with PBS/FBS/1% saponin, and incubated with 50 µl of the 1:12.5 diluted secondary antibody, goat anti-mouse IgFITC, in the dark at 4°C for 45 min. After washing, the cells were analyzed by FACScan flow cytometry using a 488-nm argon laser at predetermined instrument settings. Cell debris was excluded on the basis of its forward scatter (FSC) and side scatter (SSC) properties, and a sample of 10,000 cells was recorded for each suspension. Median fluorescence (FITC) values were determined from the FL1 histogram for unstained (isotype control) and stained cells, and the number of RAP per cell were evaluated from the standardized Quantum Simply Cellular bead (QSC; Flow Cytometry Standards Corp., San Juan, Puerto Rico) calibration plot (Zagursky et al., 1995; Li et al., 1997a). The bead standards consist of four populations of microbeads coated with goat anti-mouse antibody that bind different numbers of mouse IgG monoclonal antibody molecules (8,857, 20,094, 73,915, and 206,086 molecule binding capacity) and a blank population. QSC beads were stained in the same way as the tumor cells. A histogram of green fluorescence (FL1) was produced for the gated data and each peak of fluorescence corresponding to bead type was identified by histogram markers. The peak fluorescence channel number was taken, and a regression curve of channel number against binding capacity was constructed. A regression equation for the curve was calculated using Quickcal, the calibration software provided with the beads.

For cell surface RAP analysis, 50 µl of each cell suspension in PBS/FBS was incubated with 50 µl 7F1 at 4°C for 45 min. Further samples were incubated with the same amount of the monoclonal IgG1 isotype control. The cells were washed with PBS/FBS, incubated with goat anti-mouse IgFITC, and analyzed by FACScan flow cytometry at the predetermined RAP instrument settings. The number of cell surface RAP per cell were evaluated from the standardized QSC beads as described for total cellular RAP.

Cell Surface α_2MR/LRP Determination by Flow Cytometry

Cell surface α_2MR/LRP was measured using a quantitative cytofluorimetric method similar to that for RAP, which has been described in an earlier report [Li et al., 1997a].

Western Blotting Analysis RAP and α_2MR/LRP

Tumor cells cultured in T-80 flasks were washed three times with cold HBSS, detached using a disposable cell scraper (Costar, Cambridge, MA), and collected by centrifugation for 5 min at 500*g*. The cells were lysed in 125 mM NaCl, 10 mM Tris-HCl, pH 7.4, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride (PMSF; Sigma), 5 µg/ml leupeptin (Sigma), 5 µg/ml aprotinin (Sigma), and 1% Triton X-100 (Bio-Rad, Hercules, CA); nonsolubilized material was removed by centrifugation for 10 min at 6,000*g*. Equal quantities of protein (30 µg for α_2MR/LRP, 10 µg for RAP) were subjected to 7 % (for α_2MR/LRP) or 10 % (for RAP) sodium dodecyl sulfate-polyacrylamide gel electrophoresis

(SDS-PAGE) under nonreducing conditions. The proteins were electrotransferred to nitrocellulose membrane (Bio-Rad) using a transfer buffer of 20 mM Tris-HCl, 192 mM glycine pH 8.3 in 20% methanol. Blotted membranes were blocked with 5% nonfat milk in this buffer with 0.1% Tween-20 for 60 min at room temperature, and incubated with rabbit polyclonal anti-RAP serum or anti-α_2MR/LRP monoclonal antibody (MAb) 8G1 for 60 min at 37°C. The membranes were then washed and incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit or goat anti-mouse IgG (H+L) (Bio-Rad) at room temperature for 60 min. The immunoreactive proteins were detected using the ECL system (Amersham Life Science, Little Chalfont, England).

RESULTS

Cell Surface and Total Cellular RAP Expression

All the tumor cell lines were seeded at the same initial density of 2 x10^4 cells/cm^2, cultured for 4 days, and their RAP expression analyzed by flow cytometry. For NHEM (doubling time ~100 h), cells were seeded at a density of 2 × 10^4 cells/cm^2, and harvested at 70–80% confluency. To estimate the total cellular expression of RAP (i.e., cell surface plus cytoplasmic expression), the cultured cells were permeabilized and fixed before antibody labeling. For cell surface RAP, living cells were used, and since the anti-RAP monoclonal 7F1 cannot penetrate their cell membrane when alive, only cell surface RAP was labeled. Flow cytometric analysis of permeabilized and stained cells showed that all the cell lines including NHEM expressed RAP (Fig. 1). Most RAP was detected intracellularly in all the cell lines, whereas strong staining of cell surface RAP was also seen in MM370 and MM253c1.

Using QSC microbeads, the mean numbers of cell surface and total cellular RAP were calculated from their fluorescence intensities (Table I). All the melanoma cells and NHEM expressed total cellular RAP at similar levels, ranging from ~21,000 to ~55,000 sites per cell. MM253c1 had large numbers of RAP compared with the other cell lines. Cell surface RAP on NFF, NHEM, and melanoma cell lines MM96L, MM418c1, and A2058 were from 0 to 260 sites per cell, and below the detection threshold of flow cytometry. However, the melanoma cell lines MM138, MM253c1, and MM370 had detectable cell surface RAP, ranging from ~900 to ~4,400 sites per cell.

RAP Co-Expressed With α_2MR/LRP

We have recently reported that the five melanoma cell lines, MM370, MM138, A2058, MM253c1, and MM418c1, express cell surface α_2MR/LRP at a wide range of levels [Li et al., 1997a] and that cell culture density up-regulates expression of this receptor in the breast cancer cell line BT-20 [Li et al., 1997b]. To eliminate the effect of cell culture density on α_2MR/LRP expression on the cell lines in this work, all the cell lines were cultured under the same conditions as for RAP analysis. As shown in Table I, all the cell lines expressed cell surface α_2MR/LRP, although the levels of cell surface α_2MR/LRP varied widely ranging from ~770 to ~9,000 sites per cell. The highest level of cell surface α_2MR/LRP was found in MM370, and this cell line also had high levels of cell surface RAP. However, the levels of total cellular RAP appeared to be unrelated to cell surface α_2MR/LRP expression. As shown in Table I, NHEM expressed cell surface α_2MR/LRP at ~1,000 sites per cell, a level similar to that of MM96L and A2058, and lower than other melanoma cell lines.

Western blot analysis of lysates from the cell lines was conducted using polyclonal rabbit anti-RAP serum and the anti-α_2MR/LRP MAb 8G1. Figure 2 shows the results of Western blot analysis of NHEM and the melanoma cell lines, and demonstrate the presence of RAP and α_2MR/LRP in a pattern in agreement with the flow cytometry measurements.

Cell Culture Density Effect on RAP and α_2MR/LRP Expression

A recent study [Li et al., 1997b] has shown that expression of α_2MR/LRP is cell culture density-dependent in breast cancer cell line BT-20. To examine whether cell culture density affects RAP as well as α_2MR/LRP expression in melanoma cells, Western blot analysis was performed on two melanoma cell lines, A2058 and MM370. As shown in Figure 3, the total cellular α_2MR/LRP expression in human melanoma cell lines MM370 and A2058 was markedly increased at high cell culture density, while no difference was detected in the total cellular RAP expression between these conditions in any of the cell lines. These results suggest that

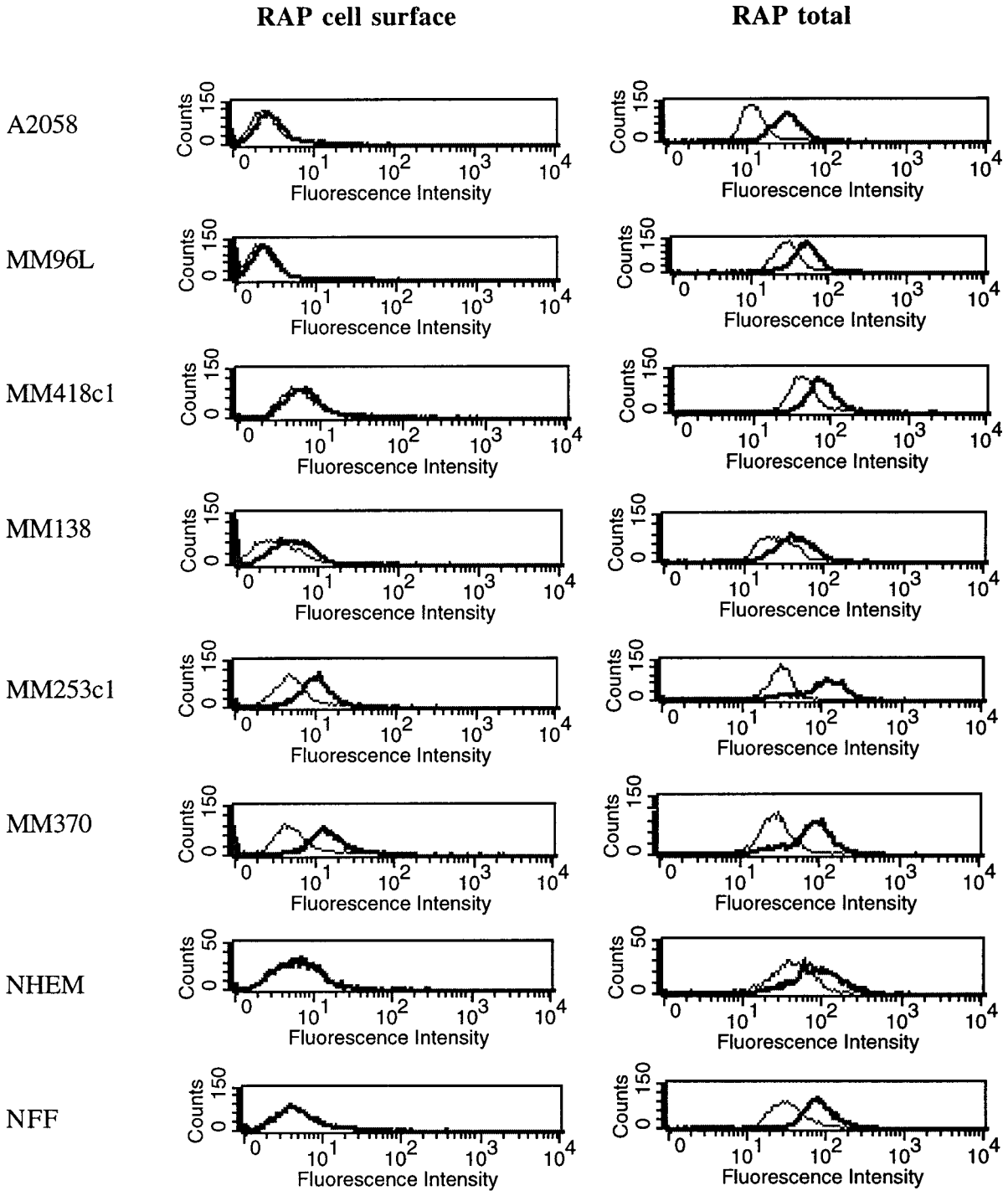

Fig. 1. Flow cytometric analysis of RAP. RAP-positive cell line NFF and melanoma cell lines were plated at the initial density of 2.0×10^4 cells/cm^2, and incubated for 4 days. NHEM was plated at the same initial density as above, but harvested at ~80% confluence (doubling time ~100 h). Cell surface and total cellular RAP was determined as described under Methods. Signals were obtained by using isotype-matched antibody control IgG1 (thin curve), and RAP monoclonal antibody 7F1 (1 μg/ml) (thick curve).

TABLE I. Number of α_2MR/LRP and RAP in NHEM and Melanoma Cell Lines[a]

Cell line	Mean number of calculated α_2MR/LRP per cell	Mean number of calculated RAP per cell		Surface/ total RAP (%)
		Cell surface	Total cellular	
A2058	767 ± 163	260 ± 60	26,545 ± 2,400	0.98
MM96L	1,148 ± 257	54 ± 48	23,426 ± 2,871	0.23
MM418c1	2,400 ± 208	0 ± 0	37,629 ± 3,311	0
MM138	3,145 ± 118	882 ± 238	21,094 ± 764	4.18
MM253c1	3,689 ± 256	3,187 ± 720	54,907 ± 7,150	5.8
MM370	8,977 ± 1,245	4,355 ± 2,110	39,706 ± 4,188	10.97
NHEM	1,027 ± 537	0 ± 0	45,105 ± 6,707	0
NFF	13,651 ± 283	0 ± 0	52,914 ± 2,815	0

[a]All cell lines were cultured and analyzed under the conditions described in Figure 1. The number of cell surface α_2MR/LRP per cell and the numbers of RAP per cell (surface or total cellular) were evaluated from QSC as described in Materials and Methods. The data shown are the mean ± SD from 4–9 determinations. The minimum detection thresholds are 72 for cell surface α_2MR/LRP, 600 for cell surface RAP, and 1,019 for total cellular RAP.

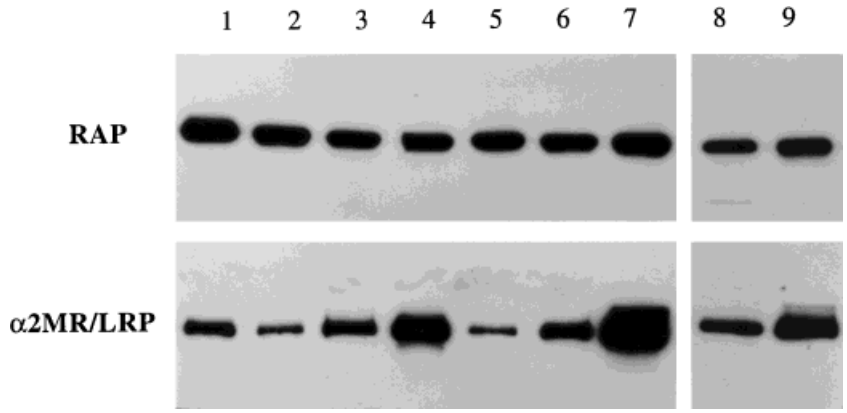

Fig. 2. Western blot analysis of RAP and α_2MR/LRP in NHEM and melanoma cell lines. NFF and melanoma cell lines were cultured under the conditions described in Figure 1. α_2MR/LRP 515 kDa and RAP in total cell lysates were detected by Western blot analysis as described under Methods. **Lane 1**, MM253c1; **lane 2,** MM96L; **lane 3,** MM418c1; **lane 4,** MM370; **lane 5,** A2058; **lane 6,** MM138; **lanes 7,9,** NFF; **lane 8,** NHEM.

the effect of high cell culture density causing an increase in the level of α_2MR/LRP expression may be a phenomenon common to many cell lines, and the α_2MR/LRP regulation is unrelated to the level of RAP expression.

DISCUSSION

In this study, we investigated the cell surface and total cellular expression of RAP using a simple and reliable quantitative cytofluorimetric technique. Previous reported techniques for cellular antigen detection used either immunoenzymatic methods or immunofluorescence. The immunoenzymatic methods, based most frequently on alkaline phosphatase, peroxidase, and avidin-biotin complexes, are mainly employed for immunohistology, where both membrane and intracellular antigens are detected but cannot be reliably differentiated. Instead, we have used immunofluorescence, which is the method of choice for investigating membrane antigens in cell suspension. The main advantage of immunofluorescence is that the analysis can be performed by flow cytometry, which allows the rapid automated investigation of large numbers of cells.

Some years ago, the first methods for flow cytometric detection of intracellular antigens were described [Slaper-Cortenbach et al., 1988; Gore et al., 1990; Drach et al., 1991]. More recently, simple methods have been described which use a commercial fixation/permeabilization solution [Pizzolo, 1994; Elson et al., 1995; Openshaw et al., 1995; Picker et al., 1995; Francis and Connelly, 1996] for detection of intracellular antigens. We have used one such product, FACS permeabilizing solution, in conjunction with 1% saponin for fixation and permeabiliza-

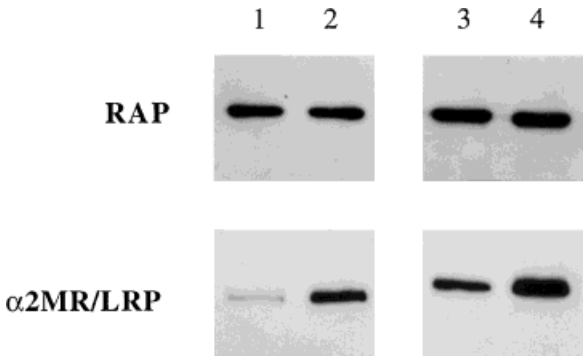

Fig. 3. Effect of cell culture density on RAP and α_2MR/LRP in melanoma cell lines MM370 and A2058. Tumor cells were plated at the intitial densities of 0.5×10^4 cells/cm^2 (**lane 1,** A2058; **lane 3,** MM370) and 8.0×10^4 cells/ cm^2 (**lane 2,** A2058; **lane 4,** MM370). Cells were incubated for 4 days, and α_2MR/LRP 515 kDa and RAP in total cell lysates were detected by Western blot analysis, as described under Methods. The result shown is one of three independent experiments with similar results.

tion. This allows a monoclonal antibody against RAP (7F1) to bind intracellular as well as cell surface RAP. For cell surface RAP, living cells (which are impervious to antibodies) were used, so that only binding of 7F1 to cell surface RAP would be measured.

Using this method, the present study clearly demonstrated that all the cell lines examined express RAP, of which by far the majority is intracellular. This result is in agreement with other reports [Strickland et al., 1991; Lundstrom et al., 1993; Orlando and Farquhar, 1993; Bu et al., 1994]. Indeed, using immunogold labeled sections, Bu et al. [1994] have shown that 94% of RAP in a human glioblastoma cell line (which expresses both α_2MR/LRP and RAP) is present in the endoplasmic reticulum (ER) and Golgi compartments, with only 2% of the total RAP being found on the cell surface. This is not unexpected, as the cDNA-deduced RAP sequence includes the putative endoplasmic C-terminal retention sequence HNEL [Strickland et al., 1991], which has been demonstrated to mediate this ER retention [Bu et al., 1995]. This fact alone would suggest that most of the RAP would be retained inside the cell.

Curiously, however, our results indicate that three melanoma cell lines express significant amounts of cell surface RAP, with two (MM253c1 and MM370) expressing the highest amounts of cell surface RAP reported to date. In the case of MM370, around 11% of total cellular RAP was found on the cell surface. The reason for such high levels on the cell surface is unknown.

It has been proposed that RAP acts intracellularly as a chaperone within the ER and Golgi, preventing the binding of ligands to α_2MR/LRP. Given this, one might expect some linkage between α_2MR/LRP activity and RAP expression. Our results indicate that the levels of RAP expression are relatively constant and seem to be unrelated to α_2MR/LRP expression. Cell culture density also had no effect on RAP expression, although it up-regulated α_2MR/LRP expression in breast cancer [Li et al. 1997b] and melanoma cell lines (MM370 and A2058).

More recently, there has been growing evidence to show that RAP binds to other ligands. Two additional RAP binding receptors have been identified which are designated as sorLA-1 and Gp95/sortilin [Jacobsen et al., 1996; Petersen et al., 1997]. In addition, Petersen et al. [1996] report that RAP is phosphorylated in vivo and can be phosphorylated/dephosphorylated in a calmodulin-dependent manner. This finding not only argues for post-translational regulation of RAP function but also implies that functional RAP must be present in cellular compartments other than the ER and Golgi.

All these observations suggest that RAP may be involved in as yet unidentified physiological functions. It may be that the high level of RAP expression on the cell surface of MM370 cells offers the potential for examining one of these roles.

ACKNOWLEDGMENTS

We gratefully acknowledge the gift of the normal fibroblast cell line and the melanoma cell lines from Dr. PG Parsons of the Queensland Institute of Medical Research, Brisbane, Queensland, Australia. Dr. Alan Schwartz of the Department of Paediatrics, Washington University School of Medicine, St. Louis, MO, kindly provided the polyclonal rabbit anti RAP antiserum. The monoclonal antibody for human α2MR/LRP was generously provided by Dr. Dudley Strickland of the American Red Cross Holland Laboratory, Rockville, MD.

REFERENCES

Ashcom JD, Tiller SE, Dickerson K, Cravens JL, Argraves WS, Strickland DK (1990): The human α_2-macroglobulin receptor: Identification of a 420-kD cell surface glycoprotein specific for the activated conformation of α_2-macroglobulin. J Cell Biol 110:1041–1048.

Battey FD, Gafvel ME, FitzGerald DJ, Argraves WS, Strauss III JF, Strickland DK (1994): The 39-kDa receptor-associated protein regulates ligand binding by the very low density lipoprotein receptor. J Biol Chem 269: 23268–23273.

Bu G, Rennke S (1996): Receptor-associated protein is a folding chaperone for low density lipoprotein receptor-related protein. J Biol Chem 271:22218–22224.

Bu G, Maksymovitch EA, Geuze H, Schwartz AL (1994): Subcellular localization and endocytic function of low density lipoprotein receptor-related protein in human glioblastoma cells. J Biol Chem 269:29874–29882.

Bu G, Geuze HJ, Strous GJ, Schwartz AL (1995): 39 kDa receptor-associated protein is an ER resident protein and molecular chaperone for LDL receptor-related protein. EMBO J 14:2269–2280.

Christensen EI, Gliemann J, Moestrup SK (1992): Renal tubule gp330 is a calcium binding receptor for endocytic uptake of protein. J Histochem Cytochem 40:1481–1490.

Coukos G, Gafvels ME, Wisel S, Ruelaz EA, Strickland DK, Strauss III JF, Coutifaris C (1994): Expression of α_2-macroglobulin receptor/low density lipoprotein receptor-related protein and the 39-kd receptor-associated protein in human trophoblasts. Am J Pathol 144:383–392.

de Vries TJ, Verheijen JH, de Bart AC, Weidle UH, Ruiter DJ, Van Muijen GN (1996): Decreased expression of both the low-density lipoprotein receptor-related protein/α_2-macroglobulin receptor and its receptor-associated protein in late stages of cutaneous melanocytic tumour progression. Cancer Res 56:1432–1439.

Drach J, Gattringer C, Huber H (1991): Combined flow cytometric assessment of cell surface antigens and nuclear TdT for the detection of minimal residual disease in acute leukemia. Br J Haematol 77:37–42.

Elson LH, Nutman TB, Metcalfe DD, Prussin C (1995): Flow cytometric analysis for cytokine production identifies T-helper 1, T-helper 2, and T-helper 0 cells within the human $CD4^+CD27^-$ lymphocyte subpopulation. J Immunol 154:4294–5301.

Francis C, Connelly MC (1996): Rapid single-step method for flow cytometric detection of surface and intracellular antigens using whole blood. Cytometry 25:58–70.

Gore SD, Kastan MB, Goodman SN, Cirin CI (1990): Detection of minimal residual T cell acute lymphoblastic leukemia by flow cytometry. J Immunol Methods 132:275–286

Herz J, Hamann U, Rogne S, Myklebost O, Gausepohl H, Stanley KK (1988): Surface location and high affinity for calcium of a 500-kd liver membrane protein closely related to the LDL-receptor suggest a physiological role as lipoprotein receptor. EMBO J 7:4119–4127.

Herz J, Kowal RC, Goldstein JL, Brown MS (1990): Proteolytic processing of the 600 kd low density lipoprotein receptor-related protein (LRP) occurs in a trans-Golgi compartment. EMBO J 9:1769–1776.

Herz J, Goldstein JL, Strickland DK, Ho YK, Brown MS (1991): 39-kDa protein modulates binding of ligands to low density lipoprotein receptor-related protein/α_2-macroglobulin receptor. J Biol Chem 266:21232–21238.

Jacobsen L, Madsen P, Moestrup SK, Lund AH, Tommerup N, Nykjaer A, Sottrup-Jensen L, Gliemann J, Petersen CM (1996): Molecular charaterization of a novel human hybrid-type receptor that binds the α_2-macroglobulin receptor-associated protein. J Biol Chem 271:31379–31383.

Jensen PH, Moestrup SK, Gliemann J (1989): Purification of the human placental α_2-macroglobulin. FEBS Lett 255:275–280.

Kancha RK, Hussain MM (1996): Up-regulation of the low density lipoprotein receptor-related protein by dexamethasone in HepG2 cells. Biochim Biophys Acta 1301: 213–220.

Kim DH, Iijima H, Goto K, Sakai J, Ishii H, Kim HJ, Suzuki H, Kondo H, Saeki S, Yamamoto T (1996): Human apolipoprotein E receptor 2. A novel lipoprotein receptor of the low density lipoprotein receptor family predominantly expressed in brain. J Biol Chem 271:8373–8380.

Kounnas MZ, Argraves WS, Strickland DK (1992): The 39-kDa receptor-associated protein interacts with two members of the low density lipoprotein receptor family, α_2-macroglobulin receptor and glycoprotein 330. J Biol Chem 267:21162–21166.

Krieger M, Herz J (1994): Structures and functions of multiligand lipoprotein receptors: Macrophage scavenger receptors and LDL receptor-related protein (LRP). Annu Rev Biochem 63:601–637.

Li Y, Wood N, Parsons PG, Yellowlees D, Donnelly PK (1997a): Expression of α2-macroglobulin receptor/low density lipoprotein receptor-related protein on surfaces of tumour cells: A study using flow cytometry. Cancer Lett 111:199–205.

Li Y, Wood N, Yellowlees D, Donnelly P (1997b): Expression of α_2 macroglobulin receptor/low density lipoprotein receptor-related protein is cell culture density-dependent in human breast cancer cell line BT-20. Biochem Biophys Res Commun 240:122–127.

Lopes MB, Bogaev CA, Gonia SL, VandenBerg SR (1994): Expression of α_2-macroglobulin receptor/low density lipoprotein receptor-related protein is increased in reactive and neoplastic glial cells. FEBS Lett 338:301–305.

Lundstrom M, Orlando R, Saedi M, Woodward L, Kurihara H, Farquhar M (1993): Immunocytochemical and biochemical characterization of the Heymann nephritis antigenic complex in rat L2 yolk sac cells. Am J Pathol 143:1423–1435.

McGarvey T, Hussain MM, Stearns ME (1996): In situ hybridization studies of α_2-macroglobulin receptor and receptor-associated protein in human prostate carcinoma. Prostate 28:311–317.

Medh JD, Fry GL, Bowen SL, Pladet MW, Strickland DK, Chappell DA (1995): The 39-kDa receptor-associated protein modulates lipoprotein catabolism by binding to LDL receptors. J Biol Chem 270:536–540.

Moestrup SK (1994): The α_2-macroglobulin receptor and epithelial glycoprotein-330: two giant receptors mediating endocytosis of multiple ligands. Biochim Biophys Acta 1197:197–213.

Moestrup SK, Gliemann J (1991): Analysis of ligand recognition by the purified α_2-macroglobulin receptor (low density lipoprotein receptor-related protein). Evidence that high affinity of α_2-macroglobulin-proteinase complex is achieved by binding to adjacent receptors. J Biol Chem 266:14011–14017.

Mokuno H, Brady S, Kotite L, Herz J, Havel RJ (1994): Effect of the 39-kDa receptor-associated protein on the hepatic uptake and endocytosis of chylomicron remnants and low density lipoproteins in the rat. J Biol Chem 269:13238–13243.

Novak S, Hiesberger T, Schneider WJ, Nimpf J (1996): A new low density lipoprotein receptor homologue with 8 ligand binding repeats in brain of chicken and mouse. J Biol Chem 271:11732–11736.

Obermoeller LM, Warshawsky I, Wardell MR, Bu G (1997): Differential functions of triplicated repeats suggest two independent roles for the receptor-associated protein as a molecular chaperone. J Biol Chem 272:10761–10768.

Openshaw P, Murphy EE, Hosken NA, Maino V, Davis K, Murphy K, O'Garra A (1995): Heterogeneity of intracellular cytokine synthesis at the single-cell level in polarized T helper 1 and T helper 2 populations. J Exp Med 182: 1375–1367.

Orlando RA, Farquhar MG (1993): Identification of a cell line that expresses a cell surface and a soluble form of the gp330/receptor-associated protein (RAP) Heymann nephritis antigenic complex. Proc Natl Acad Sci USA 90: 4082–4086.

Orlando RA, Kerjaschki D, Kurihara H, Biemesderfer D, Farquhar MG (1992): gp330 associates with a 44-kDa protein in rat kidney to form the Heymann nephritis antigenic complex. Proc Natl Acad Sci USA 89:6698–6702.

Petersen CM, Ellgaard L, Nykjaer A, Vilhardt F, Vorum H, Thogersen HC, Nielsen MS, Jacobsen C, Moestrup SK, Gliemann J (1996): The receptor-associated protein (RAP) binds calmodulin and is phosphorylated by calmodulin-dependent kinase II. EMBO J 15:4165–4173.

Petersen CM, Nielsen MS, Nykjaer A, Jacobsen L, Tommerup N, Rasmussen HH, Roigaard H, Gliemann J, Medsen P, Moestrup SK (1997): Molecular identification of a novel candidate sorting receptor purified from human brain by receptor-associated protein affinity chromatography. J Biol Chem 272:3599–3605.

Picker LJ, Singh MK, Zdraveski Z, Treer JR, Waldrop SL, Bergstresser PR, Maino VC (1995): Direct demonstration of cytokine synthesis heterogeneity among human memory/effector T cells by flow cytometry. Blood 86:1408–1419.

Pizzolo G, Vincenzi C, Nadali G, Veneri D, Vinante F, Chilosi M, Basso G, Connelly MC, Janossy G (1994): Detection of membrane and intracellular antigens by flow cytometry following ORTHO PermeaFix. Leukemia 8:672–676.

Simonsen AC, Heegaard CW, Rasmussen LK, Ellgaard L, Kjoller L, Christensen A, Etzerodt M, Andreasen PA (1994): Very low density lipoprotein receptor from mammary gland and mammary epithelial cell lines binds and mediates endocytosis of M_r 40,000 receptor associated protein. FEBS Lett 354:279–283.

Slaper-Cortenbach IC, Admiraal LG, Kerr JM, van Leeuwen EF, von dem Borne AE, Tetteroo PA (1988): Flow cytometric detection of terminal deoxynucleotidyl transferase and other intracellular antigens in combination with membrane antigens in acute lymphatic leukemia. Blood 72:1639–1644.

Strickland DK, Ashcom JD, Williams S, Burgess WH, Migliorini M, Argraves WS (1990): Sequence identity between the α_2-macroglobulin receptor and low density lipoprotein receptor-related protein suggests that this molecule is a multifunctional receptor. J Biol Chem 265: 17401–17404.

Strickland DK, Ashcom JD, Williams S, Battey F, Behre E, McTigue K, Battey JF, Argraves WS (1991): Primary structure of α_2-macroglobulin receptor-associated protein: Human homologue of a Heymann nephritis antigen. J Biol Chem 266:13364–13369.

Willnow TE, Armstrong SA, Hammer RE, Herz J (1995): Functional expression of low density lipoprotein receptor-related protein is controlled by receptor-associated protein in vivo. Proc Natal Acad Sci USA 92:4537–4541.

Willnow TE, Rohlman A, Horton J, Otani H, Braun JR, Hammer RE, Herz J (1996): RAP, a specialized chaperone, prevents ligand-induced retention and degradation of LDL receptor-related endocytic receptors. EMBO J 15:2632–2639.

Zagursky RJ, Sharp D, Solomon KA, Schwartz A (1995): Quantitation of cellular receptors by a new immunocytochemical flow cytometry technique. Biotechniques 18:504–509.

Zheng G, Bachinsky DR, Stamenkovic I, Strickland DK, Brown D, Andres G, McCluskey RT (1994): Organ distribution in rats of two members of the low-density lipoprotein receptor gene family, gp330 and LRP/α_2MR, and the receptor-associated protein (RAP). J Histochem Cytochem 42:531–542.