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Interferon-gamma upregulates MUC1 expression in haematopoietic and epithelial cancer cell lines, an effect associated with *MUC1* mRNA induction

P.K. Reddy, D.V. Gold, T.M. Cardillo, D.M. Goldenberg, H. Li, J.D. Burton*

Center for Molecular Medicine and Immunology, 520 Belleville Avenue, Belleville, NJ, 07109 USA

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Abstract

Epithelial mucin-1 (MUC1) is an important target antigen that it is overexpressed in both epithelial and haematological cancers including multiple myeloma (MM) and some lymphomas and leukaemias. MUC1 has adhesive and immunosuppressive properties, which may promote cancer progression. These studies evaluated the effect of IFNs on MUC1 expression, since these agents are widely used in clinical cancer therapy. MUC1 and interferon (IFN) receptor expression were measured by radioligand binding. Changes in *MUC1* mRNA levels in response to IFN-γ were assessed by semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR). IFN-γ was found to be a more potent inducer of MUC1 expression than IFN-α. ^{125}I-IFN binding studies indicated that both IFN receptors were expressed in most of the cell lines. With IFN-γ treatment, there was upregulation of *MUC1* mRNA. IFN-γ has a more consistent and more potent effect upon MUC1 induction than IFN-α. The ability to upregulate MUC1 across a broad range of cancer types by a clinically available cytokine, IFN-γ, has important implications for enhancing immunotherapeutic approaches targeting MUC1.

Keywords: Monoclonal antibody; MUC1; MA5; Interferon; receptor; Induction

1. Introduction

Epithelial mucin-1 (MUC1) is one member of a group of heavily glycosylated proteins known as mucins that are designated MUC1 through MUC13. These molecules are selectively localised to the apical glycocalyces of internal epithelia, and may be either secreted or membrane-bound [1–3]. This group of glycoproteins have high molecular masses and heavy, but variable, glycosylation. The corresponding mucin core proteins vary in their sequence, and several of them contain multiple variable number tandem repeats (VNTR) sequences. In earlier efforts to generate mAbs directed against epithelial cancer antigens using tumour-derived and other immunogen preparations, MUC1 proved to be quite antigenic. Thus, many anti-MUC1 mAbs have been generated, most of which recognise epitopes in the VNTR sequence [4]. *MUC1* cDNAs were shown to consist of three general regions, a 5′-upstream region, a central VNTR region and a 3′-downstream region [5–8], with a high level of nucleotide sequence identity (>99%) between cDNAs from different sources (breast and pancreatic carcinoma lines) [9]. Due to differing numbers of VNTRs, MUC1 species have MWs of 300 to >1000 kDa, with additional heterogeneity resulting from O-type glycosylation [10,11]. MUC1 is a type I

Abbreviations: A_{280}, absorbance at 280 nm; ATCC, American Tissue Culture Collection; CEA, carcinoembryonic antigen; FAB, French-American-British classification; FBS, fetal bovine serum; IFN-α, interferon-alpha; IFN-γ, interferon-gamma; JAK, janus-associated kinase; mAb, monoclonal antibody; MUC1, epithelial mucin-1, polymorphic epithelial mucin or epithelial membrane antigen; MM, multiple myeloma; NSCLC, non-small cell lung cancer; SQ-RT-PCR, semi-quantitative reverse transcriptase-polymerase chain reaction; PBS, 0.01 M sodium phosphate buffer, pH = 7.2, containing 0.15 M sodium chloride; STAT, signal transducer and activator of transcription; TAA, tumour-associated antigen; VNTR, variable number tandem repeats

* Corresponding author. Tel.: +1-973-844-7024; fax: +1-973-844-7020.

E-mail address: jburton@gscancer.org (J.D. Burton).

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membrane glycoprotein, which is glycosylated through repetitive cycling between the Golgi compartment and the cell surface. MUC1 levels increase with malignant transformation, and its distribution becomes diffuse, no longer being restricted to the apical surfaces of epithelial cells. This has both diagnostic and therapeutic implications in oncology, as this leads to shedding of MUC1 into the circulation, thus making it a useful tumour marker. In addition, this re-orientation of MUC1 allows access of anti-MUC1 mAbs to tumour sites, while they are mostly excluded from normal epithelial tissues.

While MUC1 expression is associated mostly with epithelial tissues and derivative carcinomas, its expression can be detected on some normal haematopoietic and mesenchymal cell types. It is also expressed by the majority of certain lymphoid cancers, such as multiple myeloma (MM), anaplastic large cell lymphoma, lymphocyte-predominant Hodgkin's disease and by approximately 50% of T-cell lymphomas [12–16]. Recently, the expression of MUC1 was observed in myeloid leukaemias [17], particularly those of monocytic lineage (FAB M4 and M5). Elevated levels of circulating MUC1 have also been observed in MM, with one study showing that 12/25 (48%) MM serum samples tested positive [14]. This study also demonstrated that *in vitro* cell-mediated immune reactivity versus MUC1 could be generated from peripheral blood mononuclear cells of a MM patient. Cytotoxicity was observed for $MUC1^+$, but not $MUC1^-$ cell lines. We have also shown reactivity of the MA5 anti-MUC1-VNTR mAb with MM cell lines and clinical specimens [18]. This mAb was previously shown to be reactive with $>90\%$ of clinical specimens of breast, ovarian and non-small cell lung carcinoma (NSCLC) [19].

The tumour-associated antigens (TAAs), carcinoembryonic antigen (CEA) and sialyl-T_n, have been shown to be upregulated by both interferon-alpha (IFN-α) and Interferon-gamma (IFN-γ). This was demonstrated *in vitro* in epithelial cell lines as well freshly isolated human adenocarcinoma cells, and in tumour xenografts [20–26]. Preclinical studies showed that treatment of tumour-bearing mice with IFN-γ led to increased therapeutic efficacy of radiolabelled anti-CEA and anti-sialyl-T_n mAbs [23,24]. Assessment of the effect of IFNs on the expression of these TAAs in patients with cancer who were treated with IFNs demonstrated increases of both shed (serum) and tumour-cell associated CEA and sialyl-T_n [25,26].

One prior report addressing only ovarian cancer cell lines (four in total) showed that IFN-γ increased MUC1 levels in cell extracts using an enzyme-linked immunosorbent assay (ELISA)-based approach [27]. Another study showed that IFN-γ treatment of short-term cultures of human breast cancer specimens resulted in upregulation of several cell-surface antigens including MUC1 [28]. These reports focused on selected cell lines and specimens in ovarian and breast carcinoma, respectively, without associated mechanistic studies. In addition, since the publication of these reports, an analysis of the *Muc1* 5′ promoter was reported, which showed canonical signal transducer and activator of transcription (STAT)-protein DNA-binding sequences, suggesting that IFN effects on MUC1 might be mediated by the janus-associated kinase (JAK)/STAT pathway [29]. These data provided additional support for the current studies, which assess IFN effects upon MUC1 expression across a broader range of epithelial cancer cell lines together with haematological cancer cell lines.

In the studies reported herein, the effect of both IFN-α and IFN-γ on the level of MUC1 expression across this broad panel of human cancer cell lines was assessed. We specifically addressed the questions: (1) what are the relative potencies of IFN-α and IFN-γ for MUC1 upregulation; (2) to what degree were IFN effects dependent upon the levels of the corresponding cognate membrane-bound IFN receptors; and (3) does MUC1 upregulation occur at the transcriptional level.

2. Materials and methods

2.1. Cell lines

The MM cell lines, U266 and MC/CAR, were obtained from the American Tissue Culture Collection (ATCC) (Manassas, VA, USA), as were the myeloid leukaemia and lymphoma cell lines, K562 and U937. The MM cell line, KMS12-PE, was generously provided by Dr T. Otsuki. A derivative of the HL-60 myeloid leukaemia line, HL-60MRI, as well as the NSCLC line, MV522, were generously provided by Dr M. Kelner. The breast carcinoma cell lines, MCF7, MDA-MB468 and ZR-75-30, the NSCLC lines, A549, CALU-1 and CALU-3, and the prostate carcinoma cell line, DU-145, were obtained from ATCC. The renal carcinoma cell lines were also obtained from ATCC. The ovarian carcinoma cell line, 2008, was obtained from Dr S. Howell. The ovarian carcinoma lines, SK-OV3 and SK-OV4 were obtained from Dr L.J. Old. The ovarian carcinoma lines, HEY, A2780 and OV1063 were obtained from Dr R. Baumal, Dr T. C. Hamilton and Dr A. Gabizon, respectively. The M12 and ALVA-31 prostate carcinoma cell lines were obtained from Dr J. Ware and Dr R. Ostenson, respectively. All cell lines were maintained in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS) plus antibiotics, except for the MC/CAR line, for which the media preparation recommended by ATCC was utilised.

2.2. mAbs and IFNs

The MA5 mAb is a murine IgG_1, kindly provided by Immunomedics, Inc. (Morris Plains, NJ, USA). This

mAb has been characterised previously and has been studied clinically as a radioantibody imaging agent for the detection of breast cancer [4,19]. The other anti-MUC1 mAbs that were utilised (mostly for flow cytometry) are also murine IgG_1s. These were KC-4G3 (obtained from ATCC), MA9 [4] and PAM4 mAb [30]. The first 3 mAbs react with epitopes within the VNTR region, while PAM4 reacts with an epitope that is N-terminal to and outside of the VNTR domain of MUC1.

Registered forms of IFN-α_{2a} (Roferon-A®, Roche Pharmaceuticals, Nutley, NJ, USA) and IFN-γ_{1b} (Actimmune®, InterMune Pharmaceuticals, Inc., Burlingame, CA, USA) were used for *in vitro* treatment of cell lines and for radiolabelling. A concentration range of 50–1000 U/ml of both IFNs were used for *in vitro* treatment of cells lines for durations of 24–72 h.

2.3. Radiotracer binding to cell lines

MA5 mAb was radioiodinated using $Na^{125}I$ (NEN, Boston, MA, USA). ^{125}I was activated using pre-coated Iodogen tubes (Pierce, Rockford, IL, USA), according to the method of Chizzonite [31]. Briefly, 100 μl of 0.2 M sodium phosphate buffer, pH 7.4, was used to dilute 1 mCi of $Na^{125}I$, after which this solution was transferred to a pre-coated Iodogen tube and activated for 6 min at room temperature. It was then transferred to a 1.5 ml tube containing 30 μg of MA5 mAb in 25 μl of phosphate-buffered solution (PBS) and the protein iodination reaction was allowed to proceed for 6 min at room temperature; bound and free iodine were separated on a PD-10 column (Pharmacia, Piscataway, NJ, USA). Peak protein fractions were pooled and analysed by both instant thin-layer chromatography and gel-filtration high performance liquid chromatography (HPLC), with radioactive and A_{280} monitoring, with the carrier protein, albumin, being the internal standard. ^{125}I-MA5 routinely showed $<3\%$ unbound iodide and $>95\%$ monomeric IgG with a specific activity of 444-925 MBq/mg protein (12–25 mCi/mg).

IFN-α_{2a} (Roferon®-A) and IFN-γ_{1b} (Actimmune®) were used for radiolabelling. IFNs were labelled in the same fashion as described above, using 5 μg of each of the IFNs and 1 mCi of $Na^{125}I$ per labelling reaction. For both IFNs, the $Na^{125}I$ was activated for 6 min. Since IFN-γ_{1b} labelled more readily than IFN-α_{2a}, it was reacted with the pre-activated $Na^{125}I$ for 5 min at room temperature, while the IFN-α_{2a} was reacted for 7 min at a somewhat higher pH (pH = 8). ^{125}I-IFNs routinely had $<5\%$ unbound iodide with specific activities of 888–2000 MBq/mg protein (24–54 mCi/mg). For all cell-binding experiments, 1×10^6 cells/tube in a 100 μl volume of binding buffer (RPMI 1640 medium with 10% FBS) were assayed. To each tube, either binding buffer or a 150-fold molar excess of unlabelled ligand was added. Tubes were incubated for 15 min at 4 °C, after which ^{125}I-MA5 mAb was added at a final concentration of 0.75 μg/ml for 90 min at 4 °C (saturating binding conditions). IFN binding experiments were done in similar fashion, with a final concentration of both labelled IFNs of 50 ng/ml and an incubation time of 90 min at 4 °C. Cell-bound label was separated from free label by centrifuging the cells ($7000\times g$ for 75 s) through a 200 μl cushion of 80% dibutyl phthalate/20% olive oil (Sigma-Aldrich, St. Louis, MO, USA) in 0.4 ml polyethylene tubes. The tips of the tubes were excised and counted in a gamma counter.

2.4. Semi-quantitative RT-PCR

The starting material for reverse transcriptase-polymerase chain reaction (RT-PCR) was total RNA, which was isolated from PBS-washed cells that were solubilised with a guanidine isothiocyanate-based buffer (Tri-Reagent, Sigma-Aldrich, St. Louis, MO) according to a modification of the method of Chomczinski [32]. Total RNA (5 μg) was used as the template for cDNA synthesis, employing the First Strand kit of Novagen (Madison, WI, USA), with 4% of the resulting cDNA product being used for each PCR reaction. For PCR, primers and deoxynucleotide triphosphates (dNTPs) were added at concentrations of 0.5 and 200 μM, respectively. The primers, TAC-CAA-GAG-CTG-CAG-AGA-GAC-A (forward) and TCT-TTC-GGC-GGC-ACT-GAC-AGA (reverse) were utilised, which amplify a 352 bp sequence 3′ to the *MUC1*-VNTR (GenBank accession J05582, human pancreatic mucin mRNA). Thermostable DNA polymerase (0.5 μl KlenTaq; Ab Peptides, St. Louis, MO, USA) was added to each tube and 27 cycles of PCR were carried out under the following conditions: initial denaturation of 95 °C×5 min, annealing at 56 °C×75 s, extension at 72 °C×1 min and denaturation at 94 °C×45 s. Normalisation of PCR reactions between control cDNAs and cDNA from IFN-γ-treated cells was accomplished using a QuantummRNA PCR kit (Ambion, Inc., Austin, TX, USA) that uses 18S rRNA as an internal control. The 18S primer pair, from which an amplicon of 488 bp is generated, was used.

3. Results

3.1. IFN-γ treatment increases MUC1 levels in both epithelial and haematopoietic cell lines

A panel of cell lines was screened initially for basal MUC1 expression levels by either flow cytometry using MA5 and other anti-MUC1 mAbs or by radiotracer binding with ^{125}I-MA5. Cell lines of various lineages that showed from low to high baseline MUC1 expression were then tested for their responsiveness to IFNs-α

and-γ. The panel of human cell lines that was evaluated consisted of a range of haematopoietic and epithelial cancer cell lines, including haematopoietic cell lines of both lymphoid and myeloid origin, as well as carcinoma lines of non-small cell, breast, prostate, colon, renal and ovarian derivation. A range of IFN concentrations and durations of exposure were tested, and the most consistent and greatest upregulation of MUC1 was observed with both IFNs after treatment with 250 U/ml for 48 h. The effect of IFN-γ treatment on MUC1 levels is displayed in Table 1. Overall, 20/30 of the cell lines in the panel tested showed MUC1 upregulation of >1.6 fold in response to IFN-γ, with increases of >20 fold being observed. In contrast, only 5/15 cell lines showed such a response to IFN-α ($P=0.004$; see Section 3.2 below and Fig. 1).

Table 1
Relative increases in MUC1 protein levels in response to IFN-γ

	Fold increase±S.D.[a]
Myeloid leukaemia	
HL-60MRI	10.61±2.26
K562	2.05±0.27
U937	5.47±1.43
Multiple myeloma	
ARH-77	1.49±0.24
KMS11	1.26±0.11
KMS12-PE	6.52±1.97
MC/CAR	2.89±0.85
RPMI 8226	1.24±0.34
U266	1.81±0.25
Breast carcinoma	
MCF-7	1.56±0.43
MDA-MB468	0.75±0.45
ZR-75-30	3.67±0.37
Colon carcinoma	
HCT-15	6.47±2.66
HT29	5.21±2.39
Non-small cell lung carcinoma	
A549	1.73±0.27
CALU-1	4.71±2.28
CALU-3	4.27±2.44
MV522	33.12±3.81
NCI-H322	2.45±0.06
Ovarian carcinoma	
A2780	1.91±0.37
HEY	2.54±0.77
OV1063	0.79±0.25
SK-OV3	0.91±0.46
SK-OV4	0.82±0.13
2008	2.13±0.27
Prostate carcinoma	
ALVA-31	20.96±8.17
DU-145	1.24±0.33
M12	1.27±0.23
Renal cell carcinoma	
ACHN	1.75±0.83
769-P	21.47±17.4

S.D., standard deviation; IFN-γ, interferon-gamma; MUC1, epithelial mucin-1.

[a] These values represent the mean±S.D. for $n=3$–5 experiments which measured ^{125}I-MA5 mAb binding; cells treated with 250 U/ml of IFN-γ for 48 h were compared to matched cells cultured with media alone for the time period.

3.2. MUC1 induction occurs more frequently in response to IFN-γ than to IFN-α.

Initially, 15 cell lines were evaluated for their MUC1 responses to the IFNs. The response cut-off of 1.6× was selected, since it was a point of demarcation for the dataset and was predicted to be a minimum biologically important level of response. Because of a clear trend towards more pronounced and more frequent responses to IFN-γ, an additional 15 cell lines were tested under the same conditions with this IFN. As noted, 20/30 cell lines (95% confidence interval (CI) = 47.1–82.7%) demonstrated a >1.6× increase in MUC1 in response to IFN-γ, while only 5/15 (95% CI of 2–57%) cell lines showed such a response to IFN-α. By chi-square analysis, the resulting odds ratio for the comparison of these two groups was 6.0 (95% CI = 1.46–26.33; $P=0.004$). It is also noteworthy that all 5 of the NSCLC lines that were tested showed a positive MUC1 response to IFN-γ (Fig. 1).

3.3. Specific radiolabelled IFN-γ binding is associated with MUC1 induction while specific IFN-α binding occurs frequently in the absence of MUC1 upregulation

To assess whether the difference between the potencies of IFN-γ and IFN-α for induction of MUC1 might be related to IFN receptor expression, ^{125}I-IFN binding studies were carried out. The vast majority of cell lines

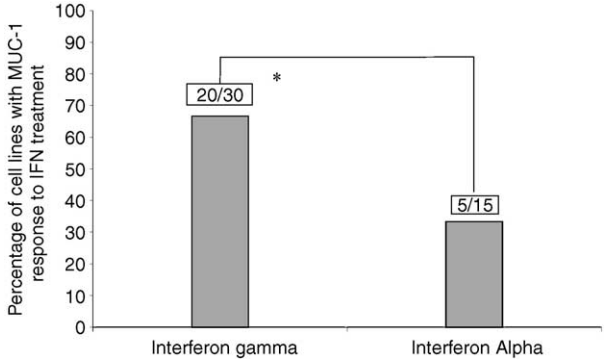

Fig. 1. A comparison of epithelial mucin-1 (MUC1) responses to interferons (IFNs) (positive response is defined as >1.6-fold increase over control). MUC1 response was evaluated in 15 cell lines via ^{125}I-MA5 mAb binding after treatment with either IFN-α or IFN-γ (250 U/ml of IFN for 48 h). After this, an additional 15 tumour lines of both haematopoietic and epithelial origin were evaluated for IFN-γ responses. The frequency of positive responses was compared between the IFN-α and IFN-γ groups by chi-square analysis, and a significant difference in MUC1 responsiveness was found with a P value of 0.004.

tested demonstrated specific binding of both IFNs. An illustrative and representative subset of the ^{125}I-IFN binding results is shown in Fig. 2. Since these studies were done to provide a preliminary assessment of whether IFN receptor levels played a major role in determining the IFN responses, formal Scatchard analysis was not performed; however, IFNs were labelled to similar specific activities, identical, saturating binding conditions were used (50 ng/ml for both IFNs) and the results have been normalised to the input cpm of ^{125}I-IFN to account for lot to lot variation in the specific activities. Of note is that the haematopoietic line, U937, showed a positive MUC1 response to IFN-γ only, despite specific ^{125}I-IFN-α binding levels that were above the median value for the cell lines tested. The other two haematopoietic lines shown, KMS12-PE and MC/CAR, both showed positive responses to both IFNs despite substantial variations in the binding levels of the two ^{125}I-IFN radiotracers. Amongst the three epithelial cancer lines shown, ZR-75-30 exhibited strong MUC1 induction in response to IFN-γ in association with high levels of specific binding of this IFN. No inductive effect of IFN-α was observed in this line, which showed lower, but reproducibly detectable, binding of ^{125}I-IFN-α. The A549 line was similarly responsive to both IFNs, despite much lower levels of ^{125}I-IFN-γ binding (a pattern similar to the MM line, KMS12-PE). Finally, the ovarian carcinoma line, SK-OV4, showed similar levels of binding both IFNs to the A549 line, but no MUC1 response to either IFN.

3.4. *MUC1* mRNA is upregulated by IFN-γ

A representative subset of the overall panel of cell lines that were tested for MUC1 induction was also assayed for changes in *MUC1* mRNA levels. Cell lines were treated with the 250 U/ml of IFN-γ for 24 h, at which time RNA was isolated and quantitated by the semi- quantitative RT-PCR method described above. As shown in Fig. 3, IFN-γ treatment resulted in increases in *MUC1* mRNA of from 1.5- to 13.3-fold. Greater mRNA induction was generally observed in the cell lines showing the greatest MUC1 glycoprotein upregulation, and this effect was observed in both epithelial and haematopoietic cell lines.

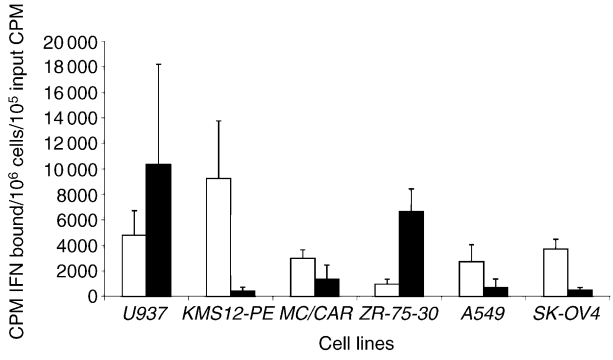

Fig. 2. Shows normalised levels of IFN binding, with specific ^{125}I-IFN-α binding shown in white and ^{125}I-IFN-γ represented by the black bars. ^{125}I-IFN-α and ^{125}I-IFN-γ of similar specific activity were tested under saturating binding conditions (50 ng/ml for 90 min @ 4° C) with and without a 150-fold molar excess of cold ligand. Specific counts per minute (CPMs) were normalised to 10^5 input cpm of ^{125}I-IFN/10^6 cells.

4. Discussion

These results demonstrate that MUC1 is upregulated upon treatment of both haematopoietic and epithelial cancer cell lines with IFN-γ. This effect of IFN-γ on MUC1 was reported previously in an *in vitro* study that was restricted to a panel of 4 ovarian carcinoma cell lines and was assessed only by an ELISA-based assay using another anti-MUC1 mAb [27]. Another group reported increases in DF3 mAb (anti-MUC1) binding after IFN-γ treatment in studies measuring changes in expression levels of several cell surface antigens in primary breast tumour cultures [28]. This report expands upon these prior studies, which had small sample sizes in two specific epithelial cancers, with the inclusion of haematological and a wider range of epithelial cancer cell lines. In addition, this report confirms these previous findings using a different anti-MUC1 mAb. This study also provides a mechanistic assessment of the inductive effect of IFN-γ upon *MUC1* mRNA levels by quantitative RT-PCR. Interestingly, the panel of six ovarian carcinoma cell lines tested in the studies reported herein did not uniformly show upregulation of MUC1. Due to the fact that these were relatively small numbers and different identities to the 4 previously reported ovarian lines [27], it is not clear what overall percentage of ovarian carcinoma lines show MUC1 induction in response to IFN-γ. In addition, while only 3 breast carcinoma lines were tested in this study, only one showed a MUC1 response to IFN-γ. Our findings suggest that MUC1 induction is seen most consistently in NSCLC lines. Since NSCLC is a major unmet need in cancer therapy, this may represent an important therapeutic lead.

This study showed that the upregulatory effect of IFN-γ on MUC1 is exerted, at least in part, through increases in steady-state *MUC1* mRNA levels. In addition, in these studies, the direct comparison of IFN-α and IFN-γ showed that IFN-γ had a more consistent and more potent effect on MUC1 expression. This did not appear to operate at the level of the cell surface receptor for IFN-α, because specific binding of this cytokine was observed in the vast majority of cell lines, including all of those that lacked a MUC1 response to this cytokine. This IFN-γ-mediated upregulatory effect on MUC1 appears to involve canonical IFN-γ/IFN-γR

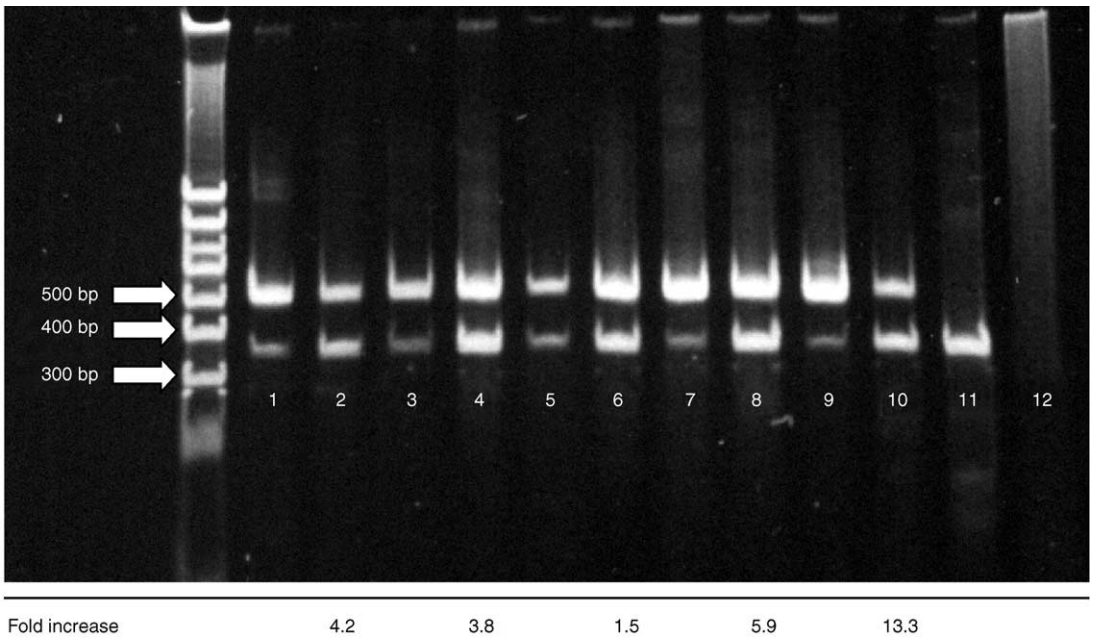

Fig. 3. A SYBR green-stained polyacrylamide gel is shown, on which equal aliquots of RT–PCR reactions were loaded. The upper band is the 18S rRNA internal control and the lower band is the MUC1 amplicon. MUC1 band intensities were quantified and normalised to the intensities of the 18S band in the same sample. On the far left are the molecular weight (MW) markers. The odd numbered lanes through lane 10 are cDNAs under control conditions and the adjacent even lanes are paired samples treated with IFN-γ. Lanes 1 and 2-HL-60MRI; lanes 3 and 4- KMS12-PE; lanes 5 and 6- U937; lanes 7 and 8-HT29; lanes 9 and 10- MV522; lane 11- SK-OV4 positive control (only MUC1 primers); lane 12- no cDNA.

signalling components starting at the exterior cell membrane along with intracellular components of the JAK/STAT-based and/or IRF-mediated IFN-γ signalling pathways. A report by Gaemers and colleagues [29] lends credence to the former mechanism, since they demonstrated functional STAT-binding sequences in the upstream promoter region of *Muc1*. In this report, we show that this transcriptional upregulatory mechanism is operative in both haematopoietic and epithelial cell lines that are responsive to IFN-γ, suggesting that control of *Muc1* gene expression is similar in both of these major cell types.

The difference observed between the effects of IFN-α and IFN-γ upon MUC1 induction suggests mechanistic differences between MUC1 and other TAAs, such as CEA and sialyl-T_n, which respond similarly to both IFNs. Prediction of the *in vivo* effects in the clinical setting is difficult to fully model due to the species-specificity of IFNs. This question requires direct testing as was done in the imaging trial in breast carcinoma with ^{131}I-CC49 mAb, which targets the TAA, sialyl-T_n [33]. In the clinical setting, the secondary induction of other cytokines related to pharmacological doses of IFNs will tend to influence the final effect on MUC1 expression. The availability of a range of registered cytokines such as IFN-α, IFN-γ, pegylated-IFN-α, as well as other cytokines with potential secondary inductive effects, such as interleukin-2 (IL-2) and granulocyte-macrophage colony stimulating factor (GM-CSF), increases the chances of finding safe and effective ways to increase MUC1 expression. The greater potency of IFN-γ for MUC1 induction is promising with regard to multimodal immunotherapeutic approaches, which could include IL-2 or investigational cytokines, such as IL-12 and IL-18, all of which strongly induce IFN-γ. The recent finding of MUC1 expression in myeloid leukaemias further expands the range of cancer indications that might be addressed through the use of anti-MUC1 mAbs in combination with immunoregulatory cytokines [16].

While tumour targeting of MUC1 with cognate mAbs should benefit from upregulation of MUC1, there are also potential adverse clinical effects related to this approach, which stem from the immunosuppressive and adhesive properties of MUC1. Upregulation of cell-bound or secreted forms of MUC1 in the absence of sufficient neutralising mAb to block attendant pathophysiological effects might, in fact, act to promote tumour growth. It is interesting to speculate as to whether this is, indeed, one of the important barriers to more effective therapy of specific MUC1$^+$ cancer types, such as renal cell carcinoma with cytokines like IL-2±IFN-α. Therapy with moderate to high-dose IL-2 alone or in combination with IFN-α induces substantial increases in serum levels of tumour necrosis factor-alpha (TNF-α) and IFN-γ, which in turn could result in significant increases in MUC1 levels. It will also be important to assess the effect of cytokine therapy on MUC1 expression by normal epithelial tissues such as lung, bladder, colon and pancreas. The expression of

MUC1 by normal epithelia is of a low level and is restricted to apical surfaces. If this orientation is maintained during cytokine therapy, any increases in MUC1 expression by these normal tissues may not be of much consequence, since mAb access would remain limited.

This IFN-γ effect upon MUC1 expression is analogous to that observed with CEA and sialyl-T_n. Regarding CEA expression, there may be somewhat greater potency for IFN-γ, but both IFNs show activity [20]. This effect also translated into a therapeutic antitumour benefit for mice treated with radiolabelled anti-CEA and anti-sialyl-T_n mAbs plus IFN-γ versus those receiving radioantibody alone [23, 24]. For IFN-α, an inductive effect was demonstrated in a clinical imaging study in which tumour uptake of ^{131}I-CC49 mAb (anti-sialyl-T_n) was compared on an intrapatient basis both before and after treatment with IFN-α. An approximate doubling of the mean tumour uptake (% injected dose/gram) of radioantibody was seen during moderate-dose therapy with IFN-α [33]. In a subsequent phase II trial in metastatic hormone-refractory prostate cancer, the combination of this radioantibody with IFN-α resulted in manageable toxicity and a suggestion of an enhanced therapeutic effect [34]. Another group used this radioantibody plus IFN-γ in a cohort of metastatic prostate cancer patients. 4 patients in this study also underwent serial tumour biopsies before and after IFN-γ treatment. Three of the four patients showed a 2–6 fold increase in the percentage of tumour cells showing positive immunostaining after IFN-γ treatment [35]. These preclinical and clinical data with other TAAs, together with findings reported herein of an IFN-γ effect upon MUC1 expression, suggest that this may also be clinically relevant and important for therapeutic strategies that target MUC1. This could have broad implications, since MUC1 is a therapeutic target for both haematopoietic and several of the major epithelial cancers.

In conclusion, this report confirms and significantly expands the scope of effects of IFN-γ on the upregulation of the TAA, MUC1. Direct *in vitro* comparison of IFN-α with IFN-γ revealed a significantly attenuated effect of the former IFN on MUC1 expression that did not appear to be related to a lack of receptors for this IFN on the cell lines tested. Moreover, it appears that the IFN-γ effect is a fundamental aspect of MUC1 regulation in that it occurs in both haematopoietic and epithelial cancer cell lines. It is not yet clear what the magnitude of this effect is in $MUC1^+$ tumour xenografts. The impact of MUC1 upregulation on tumour uptake of anti-MUC1 mAbs will be of key importance together with the overall effect on the efficacy of tumour therapy. As noted above, clinical study designs will need to take into account the fact that administration of one cytokine can induce a cascade of secondary cytokines. In any case, it would appear that IFN-γ would be the logical initial choice for $MUC1^+$ cancers, as it is a registered agent with a favourable toxicity profile and some generally beneficial immunological effects.

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