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Tyrosine phosphorylation of the MUC1 breast cancer membrane proteins Cytokine receptor-like molecules

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Abstract Phosphorylation on tyrosine residues is a key step in signal transduction pathways mediated by membrane proteins. Although it is known that human breast cancer tissue expresses at least 2 MUC1 type 1 membrane proteins (a polymorphic high molecular weight MUC1 glycoprotein that contains a variable number of tandem 20 amino acid repeat units, and the MUC1/Y protein that is not polymorphic and is lacking this repeat array) their function in the development of human breast cancer has remained elusive. Here it is shown that these MUC1 proteins are extensively phosphorylated, that phosphorylation occurs primarily on tyrosine residues and that following phosphorylation the MUC1 proteins may potentially interact with SH2 domain-containing proteins and thereby initiate a signal transduction cascade. As with cytokine receptors, the MUC1 proteins do not harbor intrinsic tyrosine kinase activity yet are tyrosine phosphorylated and the MUC1/Y protein participates in a cell surface heteromeric complex whose formation is mediated by two cytoplasmically located MUC1 cysteine residues. Furthermore, the MUC1/Y protein demonstrates sequence similarity with sequences present in cytokine receptors that are known to be involved in ligand binding. Our results demonstrate that the two MUC1 isoforms are both likely to function in signal transduction pathways and to be intimately linked to the oncogenetic process and suggest that the MUC1/Y protein may act in a similar fashion to cytokine receptors.

Key words: Breast cancer; Tyrosine phosphorylation; Receptor; MUC1

1. Introduction

Protein products of the MUC1 gene are expressed at high levels in adenocarcinomas and especially in human breast cancer tissue [1–7] and disease status in breast cancer patients is routinely assessed by monitoring the serum levels of circulating MUC1 proteins (variously referred to as episialin, H23Ag, ETA – epithelial tumor antigen, PEM – polymorphic epithelial mucin, EMA – epithelial membrane antigen, CA15–3, MCA – mammary carcinoma antigen, etc.). Molecular studies, including cDNA and gene cloning [8–14], have elucidated many properties of the MUC1 proteins. One of the MUC1 gene products is a polymorphic type 1 transmembrane molecule that consists of a large extracellular domain, a transmembrane domain and a 72 amino acid cytoplasmic tail (Fig. 1F, upper molecule). The genetic polymorphism derives from a tandem array of variable numbers of a highly conserved 20 amino acid repeat motif present within the extracellular domain. Soon after translation and prior to its translocation to the cell surface, this MUC1 protein (designated MUC1/REP) undergoes proteolytic cleavage in a region that is located 45 to 60 amino acids N-terminal to the transmembrane domain [15]. The two resulting protein molecules form a tight heterodimer complex that is composed of the large extracellular domain linked by non-covalent, SDS sensitive bonds to the much smaller (20–30 kDa) protein molecule containing the cytoplasmic and transmembrane domains [15]. Expression of the MUC1/REP protein in cell transfectants reduces cellular aggregation that is mediated by the highly glycosylated tandem repeat domain [16].

An additional novel MUC1 protein (designated MUC1/Y) has been recently characterized [8] that is devoid of the hallmark feature of MUC1, the tandem repeat array, yet retains the MUC1 N-terminal, transmembrane and cytoplasmic domains (Fig. 1F, lower molecule). The MUC1/Y protein is generated by a splicing mechanism that utilizes perfect alternative splice donor and splice acceptor sites located upstream and downstream to the tandem repeat array. Previous work demonstrated that the mature MUC1/Y protein has a molecular mass of between 42–45 kDa indicating that, in contrast to the cleaved MUC1/REP, it does not undergo proteolytic cleavage and is therefore continuous from its N-terminal extracellular domain through to its C-terminal cytoplasmic domain. In addition to the mature 42–45 kDa MUC1/Y protein, a precursor 33 kDa MUC1/Y protein that subsequently undergoes post-translational glycosylation modifications was also identified [8]. Significantly, both the MUC1/REP and MUC1/Y isoforms are highly expressed in human breast cancer tissue [1–8].

As the MUC1/REP and the novel MUC1/Y proteins are anchored at the cell surface and contain extracellular and common transmembrane and cytoplasmic domains they may both be involved in signal transduction processes.

Membrane proteins participating in signal transduction processes are in many cases modified by phosphorylation. It has not been known whether the MUC1 proteins are at all phosphorylated – indeed the MUC1 cytoplasmic domain does not contain any conserved sequence motifs known to exist in the catalytic domains of kinases in general or tyrosine kinases in particular, and is thus devoid of endogenous kinase activity and cannot undergo autophosphorylation. Similarly, cytokine receptors are also devoid of intrinsic kinase activity but are in many instances transphosphorylated on tyrosine residues by cytoplasmic tyrosine kinases [17–21]. It is shown here, for the first time, that the MUC1 proteins are phosphorylated on tyrosine residues and that following phosphorylation they have the potential to interact with SH2 domain containing proteins and thereby initiate a signal transduction cascade. Furthermore we show that, as with cytokine receptors, the MUC1/Y isoform participates in a cell-surface heteromeric complex. Interestingly, the MUC1/Y protein demonstrates sequence similarity with sequences present in cytokine receptors that are known to be involved in ligand binding. Our results demonstrate that the two MUC1 isoforms are both likely to function in signal

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transduction pathways and to be intimately linked to the oncogenetic process and suggest that the MUC1/Y protein may act in a similar fashion to cytokine receptors.

2. Materials and methods

2.1. Cells

Stable transfectants were generated by co-transfecting an expression plasmid harboring either MUC1/REP or MUC1/Y cDNA with the neomycin plasmid (pSV2 neo) selection marker into HBL100 human mammary epithelial cells (MUC1/REP transfectants) or into 3T3 *ras* transformed fibroblasts (MUC1/Y transfectants). These cells were chosen as they showed the highest expression levels of the respective MUC1 isoforms. Transient transfectants were prepared using the T7/ encephalomyocarditis virus/vaccinia sysytem (described below) and the monkey epithelial cell line, BSC-1.

2.2. cDNA constructs

The generation of expression vectors harboring either the full-length transmembrane MUC1/REP or the novel MUC1/Y cDNA and driven by the HMG coenzyme A reductase promoter (expression vector pCL642) has been previously described [8].

2.3. Transient expression of the novel MUC1 protein in the T7/EMC/ vaccinia system

Transient expression of the novel MUC1/Y protein synthesized in a state as close as possible to the naturally occurring MUC1/Y protein, was accomplished using the T7/EMC/vaccinia hybrid expression system [8]. This system utilizes the bacteriophage T7 RNA polymerase which is encoded by a recombinant vaccinia virus to transcribe genes that are regulated by the T7 promoter in the cytoplasm of infected mammalian cells. The MUC1/Y cDNA was inserted into the pTM1 vector under the control of the T7 promoter and EMCV leader, and introduced into tissue cultured BSC-1 epithelial cells together with recombinant vaccinia virus which expresses T7 RNA polymerase [8].

2.4. Western blot analyses

Cell lysates were prepared by adding lysis buffer (50 mM NaCl, 20 mM Tris-HCl, pH 7.4, 100 μg/ml leupeptin and 0.5% Nonidet P-40) to cell pellets, followed by vortex mixing and sonication (3 times 10 second bursts using a Branson sonicator). Cell debris was removed by centrifugation at 10,000 rpm for 10 min. All procedures were performed at 4°C or on ice. Protein samples were denatured by boiling in SDS buffer containing mercaptoethanol and analyzed on SDS/acrylamide gels. The gel was electrotransferred for 3 h at 1 A to nitrocellulose filters that were then blocked in PBS containing 5% skimmed milk followed by incubation with the primary antibody. The filters were washed in PBS and then incubated with a secondary anti-rabbit (or anti-mouse antibody) conjugated to horseradish peroxidase followed by ECL (Amersham) detection.

2.5. Antibodies

Western blot analyses were performed with a polyclonal antibody (a kind gift from Dr. Sandra Gendler) directed against the oligopeptide SSLSYTNPAVAATSANL (amino acids 499 to 515, see [8] for amino acid numbering) which represents the C-terminal region of the MUC1 cytoplasmic domain. The polyclonal antibody was precleared by adsorption against glutaraldehyde insolubilized human serum.

2.6. Metabolic labelling of cells with phosphate

Cells were incubated overnight in low phosphate medium supplemented with 10% fetal calf serum that had been dialyzed against saline. The following day radioactive carrier-free inorganic phosphate was added to the cells and incubation continued for another 8 h. Thirty minutes prior to harvesting, the cells were treated with the tyrosine phosphatase inhibitors, sodium vanadate (200 μM) and hydrogen peroxide (200 μM).

2.7. Immunoprecipitations

Cell lysates prepared as described above were added to protein-A-agarose-antibody complexes and incubated for 2 h at 4°C. The immunocomplex was washed 3 times with cell lysis buffer and 2× SDS sample buffer was added.

3. Results and discussion

To investigate whether MUC1 is transphosphorylated, stable transfectants expressing either the MUC1/REP protein or the novel MUC1/Y protein were generated. Immunoblotting experiments with antibodies directed against the MUC1 cytoplasmic domain confirmed MUC1/REP (20–30 kDa immunoreactive proteins) and MUC1/Y (42–45 kDa immunoreactive proteins) expression in the respective transfectants (Fig. 1A). MUC1 expressing transfectants were incubated with radioactively labelled inorganic phosphate in the presence of the tyrosine phosphatase inhibitors, hydrogen peroxide and sodium vanadate [22,23], cell lysates were then prepared and subjected to immunoprecipitation with anticytoplasmic domain antibodies. The specifically immunoprecipitated proteins migrating with molecular masses of 20–30 kDa for the MUC1/REP protein (Fig. 1B, lane 2) and 42–45 kDa for the MUC1/Y protein (Fig. 1B, lane 6) were highly labelled, indicating that the MUC1 proteins had undergone extensive phosphorylation. Similarly the MUC1 proteins were also found to be phosphorylated in non-transfected human T47D breast cancer cells (data not shown). Due however to the considerably lower level of expression as compared to that in the MUC1 transfectants, the signal of the phosphorylated MUC1 proteins in the T47D cells was correspondingly lower and further work was thus conducted with the MUC1 transfectants.

The effect of the tyrosine phosphatase inhibitors on the levels of MUC1 phosphorylation was next investigated. In the absence of these inhibitors, MUC1 phosphorylation demonstrated a low yet signficant level of phosphorylation that in their presence was markedly enhanced (Fig. 1C,D) suggesting that phosphorylation of the MUC1 proteins occurs predominantly on tyrosine residues. Consistent with this, nonspecifically precipitated labelled proteins (Fig. 1C, open arrow at left of figure) showed no differential enhancement of phosphorylation following treatment of cells with the tyrosine phosphatase inhibitors.

A phosphoamino acid analysis performed on the labelled phosphorylated MUC1 proteins showed that phosphorylation had indeed primarily occurred on tyrosine residues (70–90% in different experiments), with much reduced levels of phosphoserine and undetectable levels of threonine phosphorylation (Fig. 1E (a)). This pattern of tyrosine phosphorylation was observed both for the MUC1/REP and MUC1/Y proteins. Further confirmation for tyrosine phosphorylation of the MUC1 proteins was obtained by probing immunoblots of immunoprecipitated MUC1 proteins with antiphosphotyrosine antibodies. This analysis (Fig. 1E (b)) clearly showed that the MUC1/Y protein is readily detected by antiphosphotyrosine antibodies following treatment of cells with tyrosine phosphatase inhibitors.

Three independent lines of evidence thus support the finding that the MUC1 proteins are phosphorylated on tyrosine residues: (i) increased levels of MUC1 phosphorylation following treatment of cells with tyrosine phosphatase inhibitors, (ii) a phosphoaminoacid analysis of the MUC1 proteins, and (iii) reactivity of phosphorylated MUC1 proteins with antiphosphotyrosine antibodies.

Interestingly, tyrosine residues are distributed in a markedly biased fashion within the MUC1 proteins – 7 out of 72 of the amino acids comprising the MUC1 cytoplasmic domain are

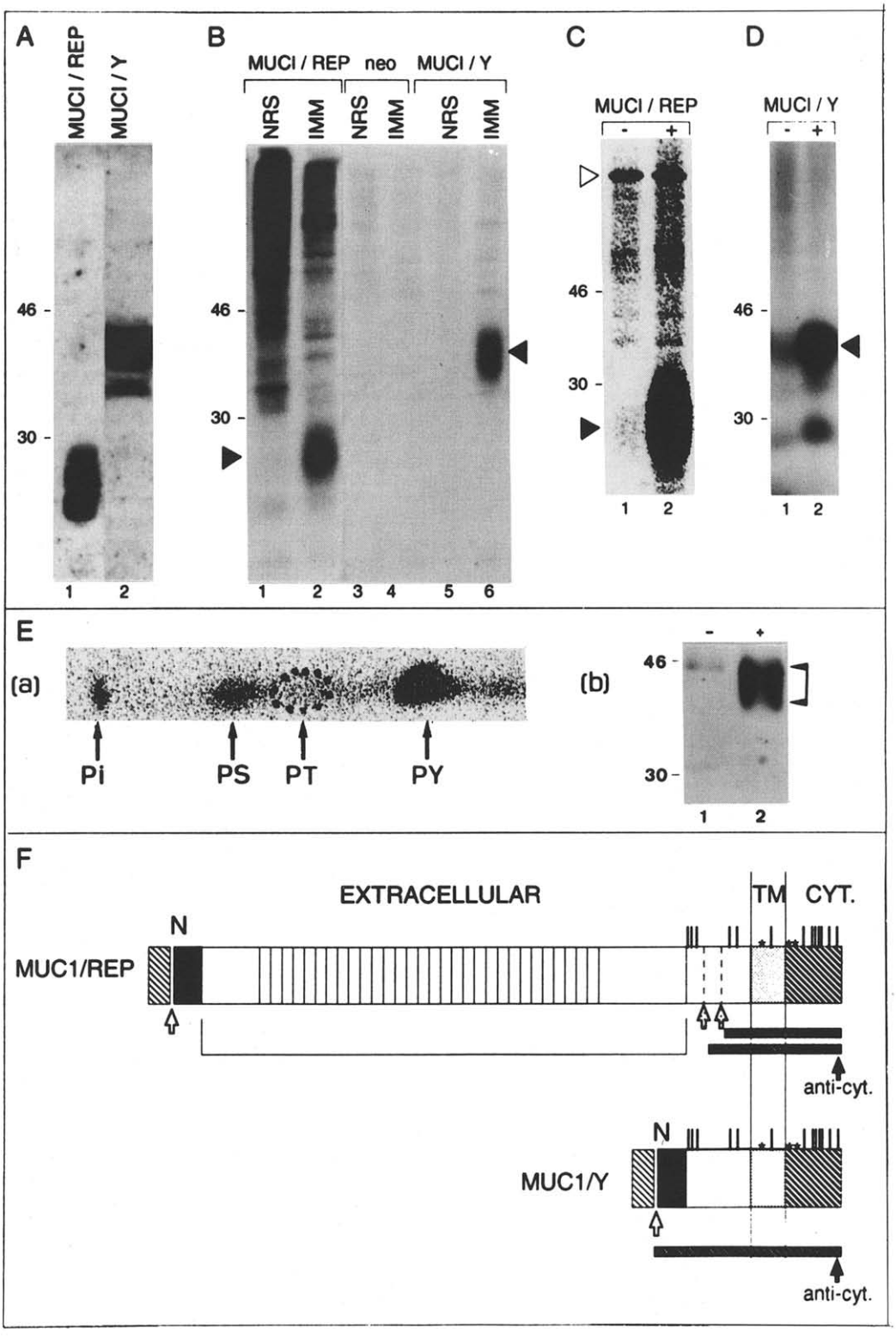

Fig. 1. Phosphorylation of the MUC1 proteins on phosphotyrosine residues. (A) MUC1/REP and MUC1/Y expression in stable transfectants. Cell lysates were prepared from the MUC1/REP (lane 1) and MUC1/Y transfectants (lane 2) and the proteins resolved on SDS-polyacrylamide (10%) gels, transferred to nitrocellulose and probed with polyclonal antisera directed against the MUC1 cytoplasmic domain, as described in section 2. Molecular size standards (in kilodaltons) are shown at left. (B) Phosphorylation of the MUC1 proteins. The MUC1/REP transfectants (lanes 1 and 2, MUC1/REP), control neomycin transfected ras 3T3 fibroblasts (lanes 3 and 4, neo) and MUC1/Y ras 3T3 fibroblast transfectants (lanes 5 and 6, MUC1/Y) were labelled with radioactive carrier-free inorganic phosphate (section 2). The cells were then harvested, briefly washed with phosphate-buffered saline and cell lysates prepared that were then subjected to immunoprecipitation with either preimmune rabbit serum (NRS, lanes 1, 3 and 5) or anticytoplasmic domain antisera (IMM, lanes 2, 4 and 6). Precipitated proteins were resolved on SDS-polyacrylamide (10%) gels and visualized by autoradiography. (C) and (D) Effect of tyrosine phosphatase inhibitors on MUC1/REP (C) and MUC1/Y (D) phosphorylation.

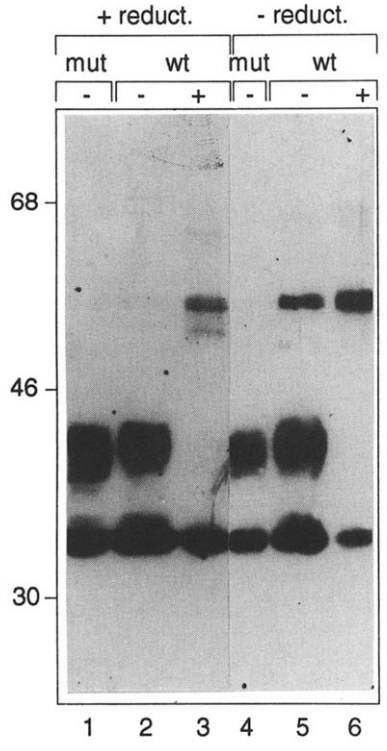

Fig. 2. The MUC1/Y protein appears in a cell surface complex, the formation of which is mediated via MUC1 cytoplasmic domain cysteine residues. Cell lysates were prepared from monkey BSC-1cells infected with recombinant vaccinia virus coding for T7 RNA polymerase and transfected with the pTM1 vaccinia expression vector harboring cDNA coding for either wild type MUC1/Y protein (wt, lanes 2, 3, 5 and 6) or mutant MUC1/Y protein (mut, lanes 1 and 4) in which the Cys-Gln-Cys (CQC) sequence had been mutated to Gly-Gln-Gly (see Fig. 1F for the location of the CQC sequence). The cell lysate proteins were resolved on SDS polyacrylamide (10%) gels under non-reducing (− reduct., lanes 4–6) or reducing (+ reduct., lanes 1–3) conditions, transferred to nitrocellulose and probed with polyclonal antisera directed against the MUC1 cytoplasmic domain. Proteins resolved in lanes 3 and 6 were derived from cells that prior to harvesting had been treated for 15 min with 15 mM EDAC crosslinking agent (obtained from Sigma). Detection of bound antibodies was performed as described in Fig. 1A.

tyrosine residues (Fig. 1F). The transmembrane domain contains one tyrosine residue and a further 5 tyrosine residues appear within the 92 amino acids N-terminally adjacent to the transmembrane domain – no additional tyrosine residues appear within the MUC1 proteins. The MUC1 amino acid sequence also reveals a marked similarity between tyrosine containing sequences located within the MUC1 cytoplasmic domain and phosphotyrosine containing peptide sequences that are *postulated* to specifically interact with SH2 domain containing proteins [26]. It should be emphasized that these sites represent only *presumptive* docking sites for SH2 domain containing proteins; it is nonetheless striking that the 72 amino acid MUC1 cytoplasmic domain contains no less than 3 such possible sites. For example, the most preferred sequence for interaction with phospholipase C γ 1 is pTyr-Val-Iso-Pro (pYVIP) and a very similar sequence [Tyr-Val-Pro-Pro (YVPP)] appears in the cytoplasmic domain of the MUC1 protein. Additionally the sequence pTyr-Glu-Glu-Val (pYEEV) which is identical to a sequence that appears within the mouse MUC1 cytoplasmic domain, has been shown to be one of the most preferred sequences for interaction with a number of SH2 domain containing cytoplasmic tyrosine kinases [26] and a potential GRB-2 binding site (pYXNX) also appears in the MUC1 cytoplasmic domain. That the MUC1 cytoplasmic domain *has the potential* to interact with SH2 domain containing proteins has been experimentally demonstrated by the binding of in-vitro tyrosine phosphorylated MUC1 cytoplasmic domain to the src SH2 domain, the SH2 domain derived from the N-terminal part of phospholipase C and to the GRB-2 protein (data not shown); no binding was observed to the SH2 domain derived from the C-terminal portion of p85 phosphatidyl inositol (PI) 3′ kinase. One should bear in mind that in-vitro tyrosine phosphorylated MUC1 cytoplasmic domain may not faithfully reflect the tyrosine phosphorylation state of this protein within the cell; experiments investigating the actual association of the MUC1 protein with SH2 domain second messenger proteins in vivo are presently being conducted. Nonetheless, the analyses described above do indicate that the tyrosine phosphorylated MUC1 protein certainly has the potential to participate in such interactions.

←
Fig. 1 (*continued*).
MUC1/REP or MUC1/Y transfectants were labelled with radioactive carrier-free inorganic phosphate as described above and either treated with sodium vanadate and hydrogen peroxide (lane 2, +) or not treated (lane1, −). Proteins immunoprecipitated with anticytoplasmic domain antibodies were visualized as above. Non-specifically immunoprecipitated proteins are indicated in (C) by the open arrow; the closed arrows indicate the specifically immunoprecipitated MUC1 proteins. (E) (a) Phosphoamino acid analysis. The immunoprecipitated phosphorylated MUC1 proteins (from lane 6 in (B)) were isolated from SDS-acrylamide (10%) gel and hydrolyzed in 6M HCl at 110°C for 1 h. Labelled phosphoaminoacids (with added unlabelled internal phosphoamino acid markers) were analyzed by thin-layer high voltage electrophoresis followed by Phosphorimager analysis. The position of migration of phosphoserine, phosphothreonine and phosphotyrosine are indicated by PS, PT and PY respectively and inorganic phosphate is shown by P_i. Phosphoamino acid analyses performed on the phosphorylated MUC1 cleavage products (lanes 1 and 2 in (C)) and the phosphorylated MUC1/Y proteins [lanes 1 and 2 in (D)] gave similar results (data not shown). (b) Reactivity of MUC1/Y protein with anti-phosphotyrosine antibodies. MUC1/Y ras 3T3 fibroblast transfectants were either treated with sodium vanadate and hydrogen peroxide (lane 2) or not treated (lane 1). Cell lysates were prepared and proteins immunoprecipitated with anticytoplasmic domain antibodies were resolved on SDS-polyacrylamide (10%) gels followed by immunoblotting and probing with antiphosphotyrosine antibodies (PY20 and PY69). The arrowed region indicates the tyrosine phosphorylated MUC1/Y protein. (F) Scheme depicting the repeat array containing MUC1 protein (upper molecule) and the novel MUC1/Y protein (lower molecule) – the novel MUC1/Y form is generated by an alternative splicing event that deletes the central tandem repeat array (compare upper and lower molecules). The location of tyrosine and cysteine residues are indicated above the rectangles by vertical lines and asterisks, respectively. Both MUC1 forms contain a hydrophobic N-terminal signal sequence (slashed box at left of figure) that is co-translationally cleaved (arrow at left of figure). This is followed by the tandem repeat array (upper molecule) that is illustrated by the block of closely spaced vertical lines. The highly hydrophobic 28 amino acid stretch constituting the transmembrane domain (TM) is shown at the C-terminal end of both MUC1 proteins, followed by the cytoplasmic domain (CYT). The region comprising the proteolytic cleavage site [15] of the repeat array containing MUC1 protein (upper molecule) is indicated by the 2 vertical dotted arrows just N-terminal to the transmembrane domain. The regions recognized by the anti-cytoplasmic domain (anti-cyt) antibodies are indicated.

Considering that within the cell less than 1% of total protein phosphorylation occurs on tyrosine residues, and that when phosphorylated, phosphotyrosine residues play a pivotal role in signal transduction processes, both the extensive tyrosine phosphorylation of the MUC1 proteins (Fig. 1) and the biased distribution of MUC1 tyrosine containing sequences (Fig. 1) that can potentially interact with SH2 domain containing proteins, are especially significant findings.

In an analogous fashion to a number of cytokine receptors [17–21,30], the MUC1 proteins, as shown above, do not have intrinsic tyrosine kinase activity yet are phosphorylated on tyrosine residues. As cytokine receptors are displayed on the cell surface as heteromeric protein complexes [17–21], we wished to investigate whether the MUC1 proteins also form cell surface heteromeric complexes. Integrity of the MUC1 extracellular domain is likely to be essential for binding of a putative ligand, and these studies therefore concentrated on the MUC1/Y isoform. Addition to MUC1/Y expressing transfectants of a crosslinking agent that does not penetrate the cell membrane lead to a substantial decline in the level of the mature cell-surface located 42–45 kDa MUC1/Y protein and concomitantly to the appearance of a new 60 kDa band (Fig. 2, lanes 3 and 6), thereby demonstrating that the MUC1/Y protein is complexed with other cell-surface located molecules. Notably, the cytoplasmically located precursor 33 kDa MUC1/Y protein was not affected by treatment with the crosslinking agent (Fig. 2, lanes 3 and 6), indicating that complex formation involved only the mature cell surface located MUC1/Y protein. It should be noted that in subsequent cross-linking experiments the 60 kDa MUC1/Y complex was consistently observed although the levels of the cell-surface located 42–45 kDa MUC1/Y protein were somewhat higher than those observed in the experiment described above – the reason for this variability is unknown. Furthermore, gel analysis of the MUC1/Y protein from cells not treated with the crosslinking agent showed, under non-reducing conditions and in the presence of iodoacetamide, a prominent 60 kDa band (Fig. 2, lane 5) that migrated to an identical position as the cross-linked 60 kDa MUC1/Y protein. This band was not observed under reducing conditions (compare Fig. 2, lanes 2 and 5), indicating that MUC1/Y complex formation is likely to be mediated, at least in part, by cysteine residues that form reducible disulfide bridges. The recently described interferon α/β cytokine receptor [31] has also been shown to form disulfide-linked dimer complexes – the cysteine residues within this cytokine receptor that are responsible for dimerization have, however, not been identified. The MUC1 protein contains only 3 cysteines – one cysteine residue appears within the transmembrane domain and the remaining two are located in a Cys-Gln-Cys tripeptide just C-terminal to the transmembrane domain (see Fig. 1F for location of cysteine residues within the complete MUC1 protein). An identical cytoplasmically located Cys-Gln-Cys tripeptide sequence has been previously shown to mediate complex formation of the cell surface CD4 molecule [32], and we thus investigated whether the MUC1 Cys-Gln-Cys sequence may play a similar role in MUC1/Y complex formation. Transfectants were generated that expressed a mutant form of MUC1/Y in which the Cys-Gln-Cys sequence had been mutated to Gly-Gln-Gly. These mutants expressed the 42–45 kDa MUC1/Y protein that migrated to an identical position as the wild type MUC1/Y protein (Fig. 2, lane 1). However, in marked contrast to the wild type MUC1/Y protein, the mutant MUC1/Y protein did not form, under non-reducing conditions, the 60 kDa complex (Fig. 2, lane 4). Additional experiments involving treatment of the mutant MUC1/Y transfectant with the crosslinking agent demonstrated only very low levels of the MUC1/Y 60 kDa complex (data not shown). This indicates that formation of the MUC1/Y 60 kDa complex is primarily mediated by the two cysteine residues present in the Cys-Gln-Cys tripeptide which form disulfide bridges, and that non-covalent protein–protein interactions may only play a minor role in complex formation. Although the molecular mass of the 60 kDa complex suggests that it is a disulfide linked heterodimer, we cannot rule out at this stage the possibility of an anomalously migrating homodimer or even a complex of more than 2 protein molecules.

It is thereby demonstrated that, as in the case of cytokine receptors, the MUC1/Y protein is also presented at the cell surface complexed to other membrane proteins. Furthermore, the MUC1 amino acid sequence reveals striking similarities to cytokine receptor sequences that are known to participate in ligand binding [33–36] (Fig. 3). For example, when considering only identical amino acid residues and Ser-Thr substitutions the

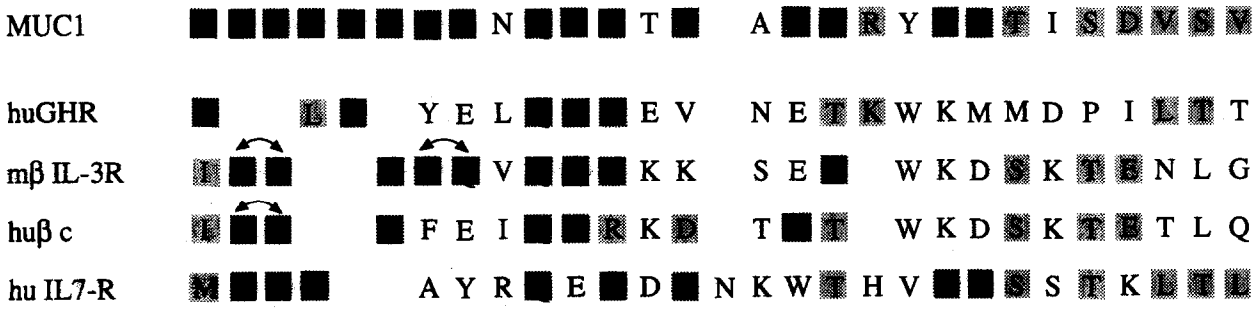

Fig. 3. Sequence alignment of residues in the MUC1 extracellular domain with the predicted ligand binding domains of cytokine receptors. The MUC1/Y extracellular domain amino acid sequence starting at amino acid number 114 [8] is compared to the predicted loop between the C-terminal B and C strands in the proposed double β-barrel structural models of the human interleukin 7 receptor (huIL-7R), β chain mouse IL-3 receptor (mβIL-3R), common β subunit of human GM-CSF (granulocyte-macrophage colony stimulating factor), IL-3 and IL-5 receptors (huβcR) and the human growth hormone receptor (huGHR). Identical amino acids are heavily shaded and conservatively substituted amino acids are lightly shaded. Note that the His-Asp (HD) dipeptide sequence that is proximal to the N-terminal of both the IL-7 receptor and MUC1 sequences appears as Asp-His (DH) both in the β chain mouse IL-3 receptor (mβIL-3R) and in the common β subunit of human GM-CSF (granulocyte-macrophage colony stimulating factor), IL-3 and IL-5 receptors (huβcR), and the Gln-Phe (QF) dipeptide that is present in the MUC1 sequence appears in the β chain mouse IL-3 receptor (m IL-3R) as Phe-Gln (FQ).

human IL-7 receptor shows 44% homology with MUC1 extracellular domain sequences over a stretch of 27 amino acids that span the ligand binding site- if one also includes in this homology comparison valine to leucine and valine to methionine substitutions, the extent of homology increases to 55%. Significantly this homology maps in close proximity to the region where proteolytic cleavage occurs in the MUC1/REP protein, suggesting that integrity of this site in the MUC1/Y protein may be of prime importance for both ligand binding and signal transmission and that different mechanisms may be responsible for activation of the two MUC1 isoforms. The MUC1/Y protein, however, contains neither the conserved extracellular domain cysteine residues nor the Trp-Ser-Xxx-Trp-Ser, motif that are characteristic of many cytokine receptors [20]- it is notable that this latter motif appears in the human growth hormone receptor as Tyr-Gly-Glu-Phe-Ser and not as the canonical WSXWS. It is therefore interesting that a Phe-Ser-Xxx-Xxx-Ser motif (Phe-Ser-Ala-Gln-Ser) does appear in the MUC1 sequence just N-terminal to the transmembrane domain, at an identical location to the WSXWS motif, seen in cytokine receptors.

Taken together, these data demonstrate that the MUC1 proteins participate in signal transduction and that the MUC1/Y protein may act as a cytokine receptor-like molecule.

It has been shown here for the first time that the MUC1 proteins are extensively phosphorylated, that phosphorylation occurs on tyrosine residues and that following phosphorylation the MUC1 proteins may potentially interact with SH2 domain containing proteins and thereby initiate a signal transduction cascade. As with cytokine receptors [17], the MUC1/Y protein does not harbor intrinsic tyrosine kinase activity yet is tyrosine phosphorylated, and participates in a cell-surface heteromeric complex – furthermore, the MUC1/Y protein demonstrates sequence similarity with sequences present in cytokine receptors that are known to be involved in ligand binding. Indeed, the striking enhancement of MUC1 phosphorylation (Fig. 1C,D) elicited by the tyrosine phosphatase inhibitors suggests the existence of regulatory mechanisms, such as ligand binding to the MUC1 extracellular domains or/and cellular redox potential changes [37] that may control MUC1 tyrosine phosphorylation levels by activating cytoplasmic kinases which subsequently transphosphorylate the MUC1 proteins. These features suggest that the MUC1/Y protein may act in a similar fashion to cytokine receptors and that following binding of an as yet unidentified ligand, undergo transphosphorylation mediated by cytoplasmic tyrosine kinases such as the Janus kinases [17,30].

Notwithstanding the fact that the two MUC1 isoforms have identical cytoplasmic domains, are phosphorylated on tyrosine residues and are both likely to participate in signal transduction processes, the difference in their extracellular domain structure and the cleavage of the MUC1/REP form as opposed to the integrity of MUC1/Y all argue against identical functions as well as activating mechanisms for the two isoforms.

We have previously shown that both the MUC1/REP and MUC1/Y proteins are highly expressed in human breast cancer tissue [8]. The elucidation of mechanisms that activate the cell-surface located MUC1 proteins, shown here to be intimately linked to signal transduction and oncogenetic processes, may lead to new modalities for the treatment of human breast cancer.

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