α-L-Fucose
A Potentially Critical Molecule in Pathologic Processes Including Neoplasia

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Abstract
α-L-Fucose is a 6-carbon deoxyhexose that is commonly incorporated into human glycoproteins and glycolipids. It is found at the terminal or preterminal positions of many cell-surface oligosaccharide ligands that mediate cell-recognition and adhesion-signaling pathways. These include such normal events as early embryologic development and blood group recognition and pathologic processes including inflammation, infectious disease recognition, and neoplastic progression. Fucosylated oligosaccharide ligands mediate cell-cell adhesion through binding to cell-surface selectins (calcium-dependent binding proteins) and calcium-dependent interactions with other cell-surface carbohydrate counterligands. A number of fucose-containing “natural ligands” are common to inflammatory and malignant cell processes. We review evidence that α-L-fucose is critically important for cell-cell and cell-matrix adhesion in a variety of normal and pathologic processes, particularly neoplasia. Current results suggest that α-L-fucose provides the essential structure that enables carbohydrate ligands to bind to selectins and to carbohydrate counterligands and thereby alter cellular homeostasis.

Recent data suggest that the sugar α-L-fucose is essential for the expression of the fully transformed phenotype in many human cell populations. Evidence for such a role comes from studies of common adenocarcinomas and Hodgkin’s disease, as well as certain melanomas, neuroblastomas, and leukemias. α-L-Fucose (hereafter denoted as fucose) is not unique or even specific to malignant tissues; in fact, fucose is incorporated into a variety of molecules, not only in humans, but also across the animal kingdom (including bacteria) and in plants. It would seem unlikely that a molecule so widely distributed should have any particular significance in cancer or other pathologic processes. Research concerned with fucose metabolism has been performed using a broad range of biologic sciences, including not only experimental oncology, but also anatomy, embryology, immunopharmacology, glycobiology, cell-adhesion biochemistry, microbiology, parasitology, and plant physiology. The breadth of studies of fucose, combined with the existence of confusing and evolving terminology among these fields, has not been conducive to the dissemination of significant results across disciplinary boundaries.

Several years ago a consistent series of intriguing results was reported from the laboratory of Carolyn Mountford, PhD, one of the pioneers in application of nuclear magnetic resonance methods to human oncology. Mountford and colleagues1 had obtained evidence, in the form of nuclear magnetic resonance spectra from malignant cells and tissues, suggesting that fucose was detectable in these cells but was limited or undetectable in nonmalignant cells from which they were believed to be derived (Figure II.1-4) More recent evidence has suggested the manner in which fucose may be used by various organisms and by human cancers. The data are incomplete and sometimes equivocal or contradictory, but the converging lines of evidence point to a specific functional
role for fucose in cell recognition and cell-matrix adhesion. For this review, results that support this hypothesis have been selected and are summarized. Much pertinent background material is only touched on since a comprehensive review of this subject would be overwhelmingly long. We hope that this overview will call attention to the key issues in the role of fucose as a biomarker that might otherwise remain obscure.

Known Distribution of L-Fucose in Biologic Systems

L-Fucose

L-Fucose (6-deoxy-L-galactose) \( \text{\textcopyright Figure 21} \) is a 6-carbon deoxyhexose and one of only a few L-sugars that are commonly used by humans in normal metabolism. In mammalian glycoproteins, only the L-enantiomer of fucose is identified.\(^5\) In these glycoproteins, L-fucose and sialic acid typically occupy terminal positions at the nonreducing ends of oligosaccharide chains. Fucose also is found in a number of membrane-associated glycolipids. There is evidence that L-fucose is accumulated in eukaryotic cells by a specific transport system.\(^6\)

A plethora of nomenclatures has tended to hinder a common understanding of the functions of these carbohydrate antigens. For example, the important minimal oligosaccharide epitope Gal\( \beta 1\rightarrow4(\text{FUC} \alpha 1\rightarrow3)\text{GlcNAc}\)\(^a\) has been reported by several different designations that have been applied in different contexts. This epitope has been known as SSEA-1 and 3-fucosyl-N-acetyllactosamine in embryologic studies, CD15 (or Leu-M1 antigen) in the parlance of hematology and surgical pathology, and Lewis X in blood banking and experimental oncology \( \text{\textcopyright Figure 31} \).\(^7\) Similarly, the carbohydrate-binding protein now known as (human) L-selectin was previously reported as LEC-CAM, LAM-1, Leu8, TQ1, and DREG-56.\(^8\) For the sake of simplicity, we designate the antigens by the Lewis antigen nomenclature whenever possible and use the selectin nomenclature to denote relevant C-type lectins.

\(^a\)In this review we use standard carbohydrate nomenclature to describe oligosaccharides (ie, Fuc for fucose, Gal for galactose, GlcNAc for N-acetyl glucosamine, and short arrows to indicate \( \alpha \)- or \( \beta \)-glycosidic linkages between the sugar residues at the carbons indicated by the respective numbers). In the interest of clarity, we avoid abbreviations for Lewis antigens, eg, Lewis Y instead of Le\(^y\), LeY or Le(y).

Fucose in Developmental Biology

Cell-surface carbohydrates participate in cell-cell recognition and cell-matrix adhesion.\(^9\)–\(^14\) These carbohydrates are
particularly important in modulating these phenomena during embryogenesis and early development. In this setting, cells are required to associate and dissociate at appropriate times for normal embryogenesis to proceed. As early as 1978, it was recognized that a small trisaccharide, expressed at the 8- through 32-cell stages of cell division, was crucial in mediating normal development in mouse embryos. This oligosaccharide was designated as stage-specific embryonic antigen-1, or SSEA-1. The structure of the minimal antigenic determinant of SSEA-1, as noted, is Galβ1→4(Fucα1→3)GlcNAc (Figure 3), identical to the Lewis X minimal antigenic determinant. Normal compaction (and subsequent embryogenesis) of the mouse embryo was inhibited in vitro by the introduction of soluble multivalent Lewis X haptens into the media during cell division and growth. Unrelated oligosaccharides had no effect on compaction. This suggested that the soluble Lewis X haptens were acting as competitive inhibitors for cell surface-bound SSEA-1 (Lewis X).16

More recently Lewis Y, a fucose-containing tetrasaccharide, has been shown to be a stage-specific antigen of 16- to 64-cell mouse embryos. Implantation is inhibited by intrauterine injection of an anti-Lewis Y monoclonal antibody (MoAb), suggesting that Lewis Y acts as a binding ligand for implantation of mouse blastocysts.17 More recent findings suggest that Lewis Y (incorporated into a membrane glycosphingolipid) mediates cell-cell adhesion by binding to a complementary carbohydrate (the H determinant), which is itself incorporated into a membrane glycosphingolipid expressed by other cells.18

**Fucose in Common Blood Group Antigens**

Fucose is a component of the H, A, and B determinants of the ABO blood group and also is found in members of the Lewis series of histo-blood group antigens, including Lewis A, Lewis B, Lewis X, and Lewis Y.19-23 The trisaccharide antigens Lewis A and Lewis X each contain 1 fucose substituent (Figure 4, A). In the lacto type 1 structure (Lewis A), the fucose moiety is attached by an α1→4 glycoside linkage to N-acetyl glucosamine (GlcNAc); in the lacto type 2 structure (Lewis X), fucose is attached by an α1→3 glycoside linkage to GlcNAc. In conventional notation, the Lewis X epitope would be described as Galβ1→4(Fucα1→3)GlcNAc (Figure 3). The tetrasaccharide Lewis antigens, Lewis B and Lewis Y, differ from Lewis A and Lewis X, respectively, by the presence of a second fucose moiety (Figure 4, B) attached in each case by an α1→2 glycoside bond to galactose.

**Fucose in Inflammation**

A large body of research supports a role for small carbohydrate ligands in trafficking of leukocytes and platelets.24-27 Specifically, the current "inflammatory cascade" model of leukocyte margination, rolling, and endothelial cell adhesion assigns a critical role to oligosaccharide ligands. The natural receptors for these ligands are believed to be carbohydrate-binding proteins now known as selectins.28-30 Selectins are members of a larger class of calcium-dependent (C-type) lectins, which require the presence of calcium, tightly integrated into a calcium-coordination site in the binding cleft, for binding of the complementary ligand.31,32 Common features of selectins include an extracellular C-type carbohydrate-recognition domain at
the amino-terminal end, an epidermal growth factor–like component, a small number of complement regulatory consensus repeats, and the transmembrane domain. A small cytoplasmic tail completes the selectin structure.

The main 3 selectin classes are located on the surfaces of endothelial cells, platelets, and leukocytes and are denoted respectively as E-selectin, P-selectin, and L-selectin. Endothelial cells also express P-selectin. The pathophysiologic role of selectins (and their complementary carbohydrate ligands) is proving to be significant in inflammatory processes of many kinds. Increased expression of E-selectin and P-selectin is now known to mediate some aspects of tissue damage in ischemia-reperfusion injury, rheumatoid arthritis, cardiac allograft rejection, diabetes, circulatory shock, inflammatory bowel disease, and graft vs host disease.\(^\text{11,12}\)

Sialyl Lewis X [Figure 4, C1] and sialyl Lewis A have been proposed as “natural ligands” for E-selectin; there is evidence that certain cell surface glycoproteins are decorated with large numbers of these ligands (and sulfated variants) in closely spaced arrangements, which may contribute to high-affinity selectin binding in vivo.\(^\text{34-37}\) Other carbohydrate ligands can serve as weak ligands for E-selectin with varying efficacy, depending on ligand structure and the relative local concentrations of these ligands and their selectin receptors.\(^\text{38}\)

**Fucose in Microbiology**

Among clinically important bacteria, *Helicobacter pylori* expresses at least 1 fucose-containing antigen (Lewis X) and also is capable of binding to the Lewis B antigen, which is expressed in normal human gastric mucosa. Current evidence suggests that Lewis B is the receptor for *H pylori* that is responsible for binding of *H pylori* to gastric mucosal cells.\(^\text{39,40}\) In the human helminth parasite *Schistosoma mansoni*, the surface of the animal during the cercarial and adult stages is covered by an immunogenic, fucose-rich glyco-calyx, and the fucose-containing antigen Lewis X is expressed in the adult parasite and in its egg.\(^\text{41,42}\) Among other microorganisms of importance to humans, *Escherichia coli* transports fucose via a specific transport (symport) protein\(^\text{43}\) and uses fucose as a precursor for its extracellular polysaccharide coat and for some of its antigenic polysaccharides.

**Fucose in Leukocyte Surface Markers**

Work performed in the field of leukocyte immunology has identified multiple markers that are expressed on the surfaces of cells and are associated with degree of differentiation—the cluster of differentiation (CD) markers.\(^\text{44,45}\) One of these, CD15, is normally expressed strongly on human neutrophils. This has, therefore, become of diagnostic usefulness in identifying myeloid-lineage neoplasms.\(^\text{46}\) It also is useful in the diagnosis of Hodgkin’s disease because the malignant cells express aberrantly high levels of the antigen not seen in normal lymphoid cells. Also, aberrantly high levels of CD15 often are expressed in adenocarcinomas but not in their morphologically mimicking mesotheliomas, so the antigen is useful in making this diagnostic distinction.\(^\text{47,48}\) The minimal antigenic determinant of CD15 is a trisaccharide with the structure Galβ1→4(Fucα1→3)GlcNAc (Figure 3).\(^\text{39-41}\) The most popular commercially available antibody to this structure has been designated Leu-M1, and CD15 is sometimes, therefore, designated as the Leu-M1 antigen.\(^\text{52}\)

**Fucose in Cancer**

Tumor-associated carbohydrate antigens (TACAs) have been studied for more than 20 years and have been the subject of numerous reviews, several of which are cited here and summarized.\(^\text{40,43-58}\) TACAs have long been designated as oncodevelopmental antigens or oncofetal antigens because many of these same antigens appear in early stages of normal development. Hakomori\(^\text{10}\) organizes the common TACAs into 3 main classes: (1) lacto-series type 1 and type 2 chains, which may be expressed on glycosphingolipids and glycoproteins; (2) core carbohydrate of O-linked mucin-type structure, expressed only on glycoproteins; and (3) precursor glycosphingolipids. The lacto-series TACAs include the common Lewis blood group antigens and hybrid antigens derived from simple Lewis antigens. These hybrid antigens include extended carbohydrate structures that are short polymers of 2 or more Lewis antigens (not necessarily of the same type), Lewis antigens to which a substituent sugar (N-acetyl-neuraminic acid, also known as sialic acid) has been added, and antigens having an extended structure and a sialic acid substituent. Some Lewis antigens may be sulfated, eg, at Gal. Lewis antigens may be expressed on glycosphingolipids and glycoproteins.

The Lewis antigens, various sialylated derivatives, and numerous hybrid or composite forms are known to be expressed, to varying degrees, in a number of human adenocarcinomas as well as in Hodgkin’s disease, certain leukemias, and malignant melanomas. Lewis X (Le\(^\text{a}\)), Lewis Y (Le\(^\text{b}\)), sialyl Lewis X (sLe\(^\text{a}\)), sialyl Lewis A (sLe\(^\text{a}\)), and the H determinant are among those expressed commonly. Hybrid or complex fucosylated antigens, which may be found in human cancers, include sialyl dimeric Lewis X, trifucosyl Lewis Y, trifucosyl Lewis B, Lewis Y-Lewis X (Le\(^\text{a}\)-Le\(^\text{b}\)), Lewis A-Lewis A (Le\(^\text{a}\)-Le\(^\text{a}\)), Lewis B-Lewis A (Le\(^\text{b}\)-Le\(^\text{a}\)), and the fucosyl-GM1 glycolipid, as well as numerous fucosylated antigens, which are less common or less well characterized.

Often there is loss of ABO antigen expression from these same malignant neoplasms concurrent with expression of Lewis antigens. Investigators have observed a relationship between loss of differentiation of malignant cells and the
appearance of additional TACAs. Expression of carbohydrate antigens is frequently more complex in metastatic lesions than in the primary tumors from which the metastases were derived. Conversely, loss of these TACAs or re-expression of carbohydrate antigens associated with normal tissue seems to correlate with successful response to treatment of the primary tumor. In general, expression of multiple TACAs or more complex TACAs is associated with poor prognosis in a number of cancers. Several of the Lewis antigens are under scrutiny as “surrogate end-point biomarkers,” which, it is hoped, will be useful in predicting the course of a malignant neoplasm in an individual patient and in guiding the choice of therapy. In addition, work is underway to target tumors with antibodies conjugated to toxins as a therapeutic maneuver. The use of BR96, directed against the Lewis Y class of antigens, is currently in early clinical trials as a therapeutic agent in human malignant neoplasms.

The other 2 classes of TACAs (as organized by Hakomori) are carbohydrate precursor structures that accumulate as a result of blocked synthesis or elongation of normal carbohydrates. The O-linked mucin-type TACAs are expressed on glycoproteins and include antigens Tn, T, sialyl-Tn, and sialyl-T. The glycosphingolipid TACAs include GM1, GM2, GM3, GD2, and GD3 among others, as well as various glycolipids that are less readily categorized. The T and Tn antigens generally do not contain fucose, although exceptions have been documented. Frequently T and Tn antigens include a sialic acid moiety. Many of the glycosphingolipid antigens similarly do not contain fucose. While these classes of antigens have important prognostic value, they are not considered further in this review.

Functions of Fucosyl-Containing TACAs

As noted, many of the fucose-containing TACAs can be identified in normal tissues in early stages of development. In such settings, they appear to function as essential mediators of cell recognition and cell adhesion and, in fact, can serve as indicators of differentiation. Several of these fucosyl-containing carbohydrates are critical in inflammatory responses as mediators of site-specific binding of circulating leukocytes to endothelial cells, platelets, and other leukocytes. Alterations in the number and distribution of cell-surface carbohydrates on neoplastic cells also have been known for many years. While the tumor-associated carbohydrate antigens have been extensively studied, an understanding of their functional significance is still evolving.

There are at least 2 general mechanisms by which fucosylated TACAs are believed to participate in cell-adhesion events. In the first, the TACA binds to a specific carbohydrate recognition site on a cell surface–receptor protein (for example, E-selectin). Recently a model for the interaction between E-selectin and sialyl Lewis X was proposed based on (1) x-ray crystallography studies of the E-selectin–sialyl Lewis X complex, (2) binding of mannose to the mannose-binding protein (a C-type lectin structurally homologous to E-selectin), and (3) the proposed conformation of the sialyl Lewis X tetrasaccharide in solution. In this model, 2 hydroxyl groups of the fucose moiety are bound directly to the calcium ion after having displaced 2 water molecules from the calcium. Similar results have been obtained from x-ray crystallography of a novel protein that mimics the carbohydrate-binding properties of E-selectin (the K3 mutant form of mannose-binding protein). The ability of fucose to function as a chelator of calcium has been shown previously. Assuming this model is correct, then an intact fucose moiety is a critical requirement for binding of a fucose-containing TACA to a C-type lectin (and therefore is also a requirement for the resulting cell-cell adhesion). Support for such a role was provided by Wright, working with Mountford and colleagues, in experiments that showed that α-fucosidase treatment nearly abolished the metastatic capability of R13762 breast carcinoma cells in a rat model. In preliminary experiments, Listinsky et al similarly found that α-fucosidase treatment reduces the invasiveness of DU-145 prostate adenocarcinoma cells in an in vitro assay.

The possible role of fucose as a calcium chelator is relevant to the second model of cell adhesion in which fucosylated TACAs are believed to participate. In this model of glycosphingolipid–glycosphingolipid interaction, cell surface carbohydrate antigens (carried on cell membrane glycosphingolipids) bind directly to other carbohydrate antigens (acting as counterligands), which also are expressed on glycosphingolipids situated on cell membranes. A possible mechanism for this interaction is suggested by x-ray crystallographic evidence that fucose can chelate calcium through sets of hydroxyl groups. The arrangement of fucose hydroxyl groups provides a favorable coordination geometry that permits them to substitute for the water molecules in the calcium hydration shell. Two fucose molecules each may chelate independently a single calcium ion, thereby cross-linking the fucose molecules in a hydrated difucose bridge based on calcium binding.

Workers in Hakomori’s laboratory found that F9 embryonal carcinoma cells (which express cell surface Lewis X antigens) autoaggregate in the presence of calcium and magnesium. This self-aggregation was inhibited by the chelating agent EDTA or by addition of the competitive inhibitor lacto-N-fucopentaose III (LFP, which includes the Lewis X epitope). In further experiments, cell-surface glycoproteins found on F9 cells were adsorbed via affinity chromatography with immobilized Lewis X glycolipid; when eluted and subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis, specific binding of the anti–Lewis X antibody SH1 was seen at approximately 100 kd, suggesting that Lewis X was expressed on these cell-surface glycoproteins. Subsequently, this same group showed that Lewis X–containing...
liposomes underwent self-aggregation in phosphate-buffered saline containing Ca\(^{2+}\) and Mg\(^{2+}\); this self-aggregation was decreased or abolished when EDTA was added to the solution. It also was shown that liposomes containing synthetic Lewis X glycolipids bound preferentially to Lewis X glycolipid that had been applied to plastic surfaces (polystyrene wells); this Lewis X–Lewis X interaction was again inhibited by LFP III and abolished by EDTA. Binding was proportional to the Lewis X density of the liposomes or amount of Lewis X coated on the polystyrene well surface. These effects did not occur when other globosides were substituted for Lewis X glycolipid.

Others since have shown that Lewis X glycolipid-coated plastic beads autoaggregate and that only Lewis X–expressing tumor cells will adhere to Lewis X–coated plates.\(^{74}\) It also was demonstrated that the major carrier of Lewis X in F9 cells ("embryoglycan") is capable of autoaggregation and that this autoaggregation is abolished when Ca\(^{2+}\) is removed by EDTA or, interestingly, when fucose is removed from embryoglycan by digestion with α-L-fucosidase. This last result is important because it is the first direct demonstration of a critical role for fucose in Lewis X–Lewis X adhesion. This finding, when taken with the calcium dependence of the process, suggests that fucose and calcium are required for Lewis X–Lewis X binding.

The binding efficacy of fucose-containing carbohydrates should be exquisitely sensitive to modifications involving the hydroxyl groups of fucose, which are believed to chelate calcium directly. There is evidence that this is true in the case of E-selectin–sialyl Lewis X binding; this evidence is considered in more detail in the following sections.

Evidence That Fucose, Not Sialic Acid, Is the Critical Sugar in Sialylated Fucosylated TACAs

Since sialic acid is frequently a component of fucose-containing TACAs, it is reasonable to ascertain whether it is sialic acid, rather than fucose, that is critical to the biologic functions of these antigens. Evidence from recent studies suggests that this is not the case. Experiments with E-selectin and its presumed natural ligand, sialyl Lewis X, have underscored 2 key features of their structure–activity relationships.

First, the sialic acid moiety is not essential under all conditions for binding of fucosylated carbohydrate chains to E-selectin or P-selectin.\(^{25-78}\) This is not to say that sialic acid is unnecessary for binding; in fact, multiple studies have shown that loss of sialic acid from the simpler fucosylated ligands (such as sialyl Lewis X) does, in fact, abolish binding. What does seem to be true, however, is that a number of more complex or extended fucosylated ligands are capable of mediating binding of neoplastic cells to E-selectin and that this binding can be accomplished via carbohydrate epitopes that do not include sialic acid. These exceptions suggest that sialic acid is not an absolute requirement for binding of fucosylated ligands to E-selectin.

Second, it has been shown that modifications of the functional groups of sialic acid (such as loss of the glycerol side chain), or replacement of sialic acid by a sulfate group, can be carried out with little or no loss of efficacy for binding to E-selectin or P-selectin in vitro.\(^{34,79-81}\) Conformationally restricted analogs of sialyl Lewis X (sialyl Lewis X mimics) have recently been synthesized and have been shown to be effective selectin-blocking agents in vitro and in vivo. An especially effective class of such blocking agents (fucosides of glycyrrhetinic acid) requires an intact fucose moiety.\(^{82}\) The portions of these blocking agents that presumably substitute for sialic acid are structurally quite different from sialic acid, lacking the nitrogen and several of the functional groups found in this molecule. It seems that for a number of the fucose-containing TACAs studied to date, an intact fucose moiety is required for biologic activity, while in contrast the sialic acid moiety often can be substantially modified or replaced with little or no effect on activity.\(^{38}\)

The Case for Fucose as a Key Participant in Malignant Behavior

It is clear that the fucoligands* as a class of cell-associated molecules are not unique to the setting of malignant transformation or progression. Rather, these oligosaccharides are developmentally or histologically ectopic when expressed by malignant cells. That is to say, for many tissues, fucoligands are ordinarily expressed in a transient fashion at a very early stage of development, and fucoligand expression after malignant transformation is inappropriately late for the degree of differentiation of the adjacent normal tissue. In some cases, the expression of fucoligands also may be inappropriate for the particular tissue type regardless of stage of development. There are exceptions: some mature and normally differentiated tissues express fucoligands,\(^{83,84}\) and some of the compound carbohydrate antigens are expressed rarely or not at all in any stage of a tissue's normal development.\(^{85-91}\) Nevertheless, for the commonly studied fucoligands (particularly Lewis X, Lewis Y, and various sialylated forms) their expression in malignant tissue can be said to be in the wrong place, at the wrong time, or both.

Current theories of metastatic spread define several steps in the metastatic cascade that all must be completed for a metastatic focus to survive and proliferate.\(^{92-96}\) This paradigm is conceptually quite similar to the currently-accepted inflammatory cascade model of leukocyte trafficking and extravasation.\(^{24-27}\) One of the key steps in metastasis via hematogenous routes is the initial binding of the circulating malignant cell to the endothelium or its basement membrane after proliferation, angiogenesis induction, and invasion.\(^{97,98}\) It is at this step that fucoligands seem most likely to participate.

If inappropriately expressed fucoligands have an important role in cell adhesion events associated with metastasis or...
even earlier during invasion, then those occasions should require the integrity of each of the steps that make the binding event possible. It follows that specific interventions that interfere with the structure or concentration of the fucoligand should predictably alter binding efficacy and that steps that increase or decrease the concentrations of the other participating molecules should, likewise, modulate the efficacy of binding. Experimentally, the issues are similar to those encountered in "proving" the role of a putative neurotransmitter or a putative receptor, except that the nature of the "receptor" in neoplastic cells is more broad and variable (including glycoproteins, glycolipids, carbohydrate counterligands, and calcium ions, as well as the clustering of fucoligands or their receptors on cell surfaces). Also, the structure-activity relationships that govern these interactions frequently are less stringent than those for conventional receptors, and more than one adhesion mechanism may be involved in any given model.99,100

We believe that the role of fucose in adhesion processes that are critical to maintain the transformed phenotype is supported by several lines of evidence. The references are keyed to the idealized summary diagram shown in Figure 51. 

Presence, Synthesis, Localization

Fucoligands should be present in the cells of interest, and they should be shown to be expressed at the cell surface. Enzymes required for synthesis of fucoligands should be present in the cells. Fucoligand expression on tumor cells should be abundant at the cells that are involved in a cell-contact or cell-adhesion process (i.e., metastasis or invasion). If distribution of fucoligands on the cell surface is not uniform,101 then fucoligands should be concentrated at membrane sites that are involved in metastasis or invasion.

Evidence

Cell-surface fucoligands have been demonstrated in most common human malignant neoplasms, including carcinomas of the colon,59,88,102 breast,103-105 ovary,106-108 prostate,109-111 lung,91,112-114 stomach,115-117 pancreas,118-121 endometrium,122-124 kidney,125-127 bladder128-130 and thyroid,131-133 as well as in Hodgkin’s disease.52,134,135 In selected cases, melanomas,136-138 neuroblastomas,139-141 hepatocellular carcinomas,142-145 carcinomas of skin,146-148 and certain leukemias51,149-152 also may demonstrate fucoligands associated with their cell membranes.

The enzymatic addition of fucose ordinarily is the last step or next to last step in synthesis of fucoligands from their precursor molecules.8,23,58 Fucose usually is present at the nonreducing end of the oligosaccharide; fucose may alternatively or additionally be attached in a subterminal position on the epitope. The fucosyltransferase enzymes of malignant cells typically catalyze the attachment of fucose to precursor carbohydrates via α1→3 or α1→4 glycosidic bonds and somewhat less frequently via α1→2 bonds. Fucosyltransferase activity has been shown to be increased in a number of human malignant neoplasms compared with normal tissues,124,132,153 and certain specific fucosyltransferase isoenzymes may be up-regulated as well.63,123,151,154-156

Immunohistochemical staining for the fucoligand sialyl Lewis X has been compared in primary colorectal carcinomas from patients with Dukes Stage D (with liver metastases) and Dukes stage C disease. Staining of the primary lesion was seen more frequently in the more advanced tumors, and incidence of sialyl Lewis X expression was greatest at cells of the invading front that showed morphologic features interpreted as focal dedifferentiation.157 The pattern of CD15 (Lewis X) expression was studied by immunohistochemistry in a series of 98 breast cancer tumors. CD15 expression was associated with the leading edge of invading tumor or with cancer cells (believed to be metastasizing) found in intravascular sites in tissue sections.105 The human breast carcinoma line 3396, studied in an in vitro model of migration, displays fucoligands concentrated most heavily at the membrane ruffles and microspike structures that are known to be associated with the cell migration process.158 Fucoligands are expressed in metastatic cells derived from the aforementioned common primary malignant neoplasms. Often the metastatic cells differ from the primary tumor cells in exhibiting patterns of fucoligand expression that are different (in intensity, complexity, or both) from the primary tumor. This trend has been interpreted as evidence of dedifferentiation or tumor progression and has been associated with poor prognosis.10,55,153,159-161

Relationship Between Fucoligand Synthesis and Adhesion

Actions that increase or decrease the synthesis of fucoligands in neoplastic cells should respectively enhance or
diminish cell adhesion and other properties associated with the neoplastic phenotype.

Evidence

Cells from human tumor cell lines HL60 (promyelocytic leukemia), Colo205 (colonic adenocarcinoma), and U937 (histiocytic lymphoma) express fucoligands of varying complexity. Cells of these 3 lines will adhere to activated human umbilical vein endothelial cells (HUVECs), which express E-selectin after activation by interleukin-1 (IL-1). Inhibition of O-glycosylation in tumor cells (by pretreatment with benzyl-α-GalNac) essentially abolishes tumor cell adhesion to activated HUVECs for all 3 cell lines. The same workers also performed a series of experiments in which E-selectin was purified and immobilized on plastic plates. This immobilized E-selectin also supported adhesion by the 3 tumor cell lines, and again, the adhesion was essentially abolished when the tumor cells were pretreated with the O-glycosylation inhibitor.

Certain nonmetastatic variants of murine tumor cell lines have been shown to lack fucosyltransferase activity. Results obtained in knockout mice provide direct evidence that fucose is an essential component of selectin ligands. Mice deficient in α1,3 fucosyltransferase Fuc-TVII exhibit a leukocyte adhesion deficiency characterized by absent leukocyte E- and P-selectin ligand activity and deficient high endothelial venule L-selectin ligand activity. The importance of fucoligands and E-selectin in metastatic disease has been studied by using transgenic mice that constitutively express cell-surface E-selectin in all tissues. Murine B16F10 melanoma cells lack cell-surface carbohydrate ligands for E-selectin; the transgenic mice develop nodular tumors only in the lung after intravenous injection of these melanoma cells. B16F10 melanoma cells can be caused to express E-selectin ligands (including sialyl Lewis A) on their surfaces by transfection with complementary DNA (cDNA) for α1,3/1,4 fucosyltransferase; injection of these transfected cells produces massive, rapidly growing liver tumors with diffuse infiltration of melanoma cells into the hepatic parenchyma.

Retinoic acid treatment of 2 rat colon carcinoma cell lines of different tumorigenic potential resulted in a decrease in α1→2 fucosyltransferase activity in the more tumorigenic variant and decreased expression of fucosylated membrane glycoconjugates in parallel with an increase in sensitivity to lymphokine-activated killer cell-mediated lysis, with no significant effect on the less tumorigenic cell line. A similar result was shown when human colon carcinoma cell lines were treated with retinoic acid. Contrary results have been obtained in other models, however. Retinoic acid–induced differentiation of F9 embryonal carcinoma cells has been accompanied by increased fucosyltransferase activity, as well as by increased fucosylation of certain specific high molecular weight cell-surface glycoproteins. Similar effects were seen with retinoic acid treatment of murine melanoma cells. The meaning of these discrepant findings is unclear. It is possible that increased differentiation may cause a redirection of fucose utilization, with relatively less incorporation of fucose into known fucoligands.

Structure-Activity Relationships

Specific modifications of fucoligands should modulate the efficacy of binding events. It follows that fucoligand analogs with isolated modifications of the fucose moiety should demonstrate profound changes in binding activity compared with authentic fucoligands.

Evidence

Wright et al have published data showing that the high metastatic potential of the R13762 rat mammary carcinoma cell line was essentially abolished in vivo by pretreatment of cells with α-fucosidase before subcutaneous injection. In a study of lymphocyte binding to high endothelium in vitro, several synthetic multivalent sialyl Lewis X glycans were shown to be potent inhibitors of lymphocyte adhesion. In contrast, fucose-free analogs having the same charge and approximately the same size as the corresponding sialyl Lewis X glycans had no effect on lymphocyte binding. Two groups have further independently shown that the ability of fucoligand mimics to compete with sialyl Lewis X for binding to E-selectin is completely eliminated by replacement of any one of the hydroxyl groups attached to carbons 2, 3, or 4 of fucose. These strict requirements also hold for L-selectin; in the case of P-selectin, only the 3-hydroxyl is absolutely required for fucoligand binding. The critical roles of the 2-, 3-, and 4-hydroxyl groups are supported by recent pharmacophore search studies of synthetic fucoligand mimics. As noted, in the case of sialyl Lewis X, the structural requirements for the sialic acid group are much less critical: major modifications in the sialic acid moiety can be carried out with little or no reduction in binding. For example, the sialic acid group can be replaced by a sulfate group or a carboxylate-bearing side chain that parenthetically may result in increased binding efficacy.

Antibody Specificity

Monoclonal antibodies that are highly specific for a particular fucoligand must require fucose as a part of the epitope.

Evidence

Competition experiments with neoglycoproteins and neoglycolipids from a variety of malignant neoplasms have been used to deduce the epitopes that are recognized by fucoligand MoAbs. In these cases, fucose is required to achieve binding. In thin-layer chromatography of gangliosides from the SW2 cell line of
small cell lung carcinoma, immunostaining with the selective MoAb SM1 is abolished by pretreatment with α-fucosidase. The authors concluded that α-fucose was the most important sugar moiety for recognition by SM1 antibodies. Fucose is considered the immunodominant sugar of the carbohydrate antigens Lewis A, Lewis B, Lewis X, and Lewis Y and of the H blood group determinant.

Requirement for Fucoligand Receptor

The fucoligand “receptor” should be present on the surfaces of the specific cells that are the targets of the fucoligand binding process. Agents that interfere with fucoligand binding should interfere with the cell-cell binding event.

Evidence

Hematogenous spread of malignant cells involves the binding of invading cells to endothelial cells or platelets. The calcium-dependent carbohydrate-binding proteins E-selectin and P-selectin are expressed on the surfaces of activated venous endothelial cells, and P-selectin is expressed on platelets. E-selectin is present on cell surfaces of activated HUVECs; HL60 leukemia cells (which express cell surface sialyl Lewis X) bind to HUVECs in vitro as noted. E-selectin in truncated form can be immobilized on an inert solid support; this immobilized E-selectin also binds HL60 leukemic cells. Specific MoAbs that are directed at the appropriate selectin (or selectins) will block binding of cells that express fucoligands.

In the case of Lewis X to Lewis X adhesion described earlier, the presence of the Lewis X antigen on the target cell, plastic bead, or polystyrene well is sufficient for binding to occur. If LFP III is added to the medium in which Lewis X to Lewis X binding is to occur, the adhesion events are reduced. In vitro, changing the level of Lewis X on the target particles leads to corresponding changes in binding. If a calcium chelator, such as EDTA, is added to the medium, Lewis X to Lewis X binding is abolished.

Relationship Between Fucoligand Receptor

Synthesis and Adhesion

Actions that alter expression of receptors for fucoligands should enhance or reduce the binding of fucoligands to the target cells.

Evidence

Transgenic mice that constitutively produce E-selectin develop large, infiltrating, rapidly growing metastatic lesions in the liver after tail vein injection of melanoma cells that express fucoligands (on stable transfection with cDNA for (αL3/1,4) fucosyltransferase). In contrast, transgenic mice that express only a truncated soluble form of E-selectin develop only a few small noninfiltrating hepatic metastases after injection of transfected melanoma cells. Antimetastatic prostacyclins that block the expression of E-selectin also inhibit the adhesion of colon carcinoma cells to cytokine-stimulated cultured human microvascular endothelial cells. Along these same lines, MDA-MB-231 breast cancer cells induce the expression of E-selectin by HUVECs; this induction effect is blocked by several corticosteroids with known antimetastatic effects and by medroxyprogesterone acetate, with inhibitory effects proportional to drug concentrations.

The specific transcription factor, nuclear factor κB (NF κ B), controls expression of a number of genes, including those related to E-selectin; NF κ B is activated by IL-1. Adhesion of tumor cells to IL-1-activated HUVECs can be inhibited by anti-NF κ B reagents, such as N-acetyl L-cysteine, aspirin, or pentoxifylline. IL-1-mediated experimental metastases (from A375M human melanoma cells) can be established in vivo in nude mice; these IL-1-mediated metastases are blocked by a recombinant human IL-1 receptor antagonist. Incubation of endothelial cells in vitro with IL-1 leads to
augmented expression of E-selectin by the cells and increased adhesion of A375M human melanoma cells. Both of these effects are inhibited when the interleukin-blocking agent IL-1 receptor antagonist is added to the incubation with IL-1.

Fucose in Cancer Cell Adhesion: Implications

As we hypothesize that fucose is essential in cancer cell adhesion for most common malignant neoplasms, a reasonable goal is to provide evidence for all of the steps shown in the idealized diagram in Figure 5. For some common fucoligands of interest, evidence currently remains incomplete for one or more of these steps. Nevertheless, the available evidence clearly implicates fucose as a likely participant in cell adhesion that underlies the spread of many human cancers. The most nearly complete evidence is from the work performed with sialyl Lewis X and sialyl Lewis A, available in part because of the activity that seeks to understand the inflammatory process.

The view that antiadhesive therapy could block metastatic spread of cancer has been expressed over the years in authoritative reviews and original investigations in glycobiology.102,27,37,97 The stringent requirement (for E-selectin binding) of an immutable fucose moiety in sialyl Lewis X contrasts with the rather loose requirements for an intact sialic acid structure in the ligand-selectin recognition process. These findings raise an interesting possibility, ie, that distant adhesion of metastatic cancer cells could be abrogated by the modification of a single hydroxyl group of fucose on the fucoligands. Obviously, strategies that modify the fucose moieties on malignant cells must consider the effects on normal inflammatory processes. One group78 has shown that binding of the MoAb Mbr8 to colon cancer cells also blocks the binding of these cells to activated endothelial cells. This same Mbr8 MoAb (directed against Lewis fucosylated type I carbohydrate chains) did not bind to polymorphonuclear leukocytes (PMNs) or lymphocytes and did not inhibit PMN binding to activated endothelial cells. These results suggest that it may be possible to simultaneously achieve 2 worthwhile objectives: (1) reducing adhesive effectiveness of malignant cells via chemical modifications of their fucoligands, while (2) allowing PMNs to bind normally to E-selectin, so that the host retains the capacity to mount an inflammatory response to an appropriate stimulus.

It may ultimately prove more expedient to use competitive inhibitors of fucoligand binding, an approach that is under investigation for anti-inflammatory purposes.100,171 One antiadhesive strategy using modified citrus pectin,183 although not known to involve fucose, shows that this approach is technically feasible. Another possibility would be to selectively inhibit one known to involve fucose, shows that this approach is technically feasible. Another possibility would be to selectively inhibit one of these steps.

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