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Commentary

Oligopeptide Transporters as Putative Therapeutic Targets for Cancer Cells

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Two recent publications have described the expression of functional oligopeptide transporters in cancer cells. The first showed that a particular fibrosarcoma cell line expresses an apparently novel transporter (1). The second study demonstrated high expression levels of the PepT1 oligopeptide transporter in two pancreatic adenocarcinoma cell lines (2). Demonstration of oligopeptide transporters expressed at the surface of two different types of cancer cells is somewhat surprising because the cell types from which these cancers were derived, fibroblasts and pancreatic duct epithelia, are not known to express significant amounts of functional oligopeptide transporters. Although it would be premature to make far-reaching conclusions from these two studies, the observations described open up the possibility of a new approach for selectively targeting anti-cancer therapeutics.

Up to this point, antibodies directed against specific, frequently overexpressed, antigens at the surfaces of cancer cells have been the primary method of targeting to cancer cells (3). Toxins or radionuclides have been conjugated to these targeting antibodies to improve their killing capacity (4). A number of approaches have been developed using targeting antibodies to deliver packaged forms of low molecular weight anti-cancer agents which typically, by themselves, produce horrendous side effects due to their inability to discriminate between normal and cancer cells (5). The potential for targeting low molecular weight anti-cancer compounds, by themselves, has been limited by the absence of a putative target or uptake pathway which required for this selective delivery.

Similar to previous approaches, an antibody against a surface epitope of these peptide transporters could be used for tumor targeting. More likely, however, is the prospect of a new class of low molecular weight anti-cancer agents which could function as substrates for the expressed transporter. These compounds, which would have direct access to the cytoplasm of the cell, might be designed to either modulate some cancer-specific metabolic event to slow the cancer growth or be converted once inside the cell into a toxic agent capable of inducing cell death. In a more elaborate scheme, antibodies which target the transporter, or some other cancer cell-specific surface epitope, could be conjugated to an enzyme which locally converts a non-substrate prodrug into a toxic compound capable of entering the cell through the peptide transporter. While multiple scenarios would seem possible, it would be foolish to get ahead of ourselves.

First, we don't know if functional oligopeptide transporters are commonly expressed in cancer cells. The cancer cells demonstrated to express functional peptide transporters are derived from tumors but have been propagated in culture and could represent clonal expansions that are uncharacteristic of the original tumor or tumor cells in general. Probes to assess the extent and distribution of oligopeptide transporter expression in cancers have only recently become available. We do know that cancer cells demonstrate altered patterns of gene expression and are more metabolically active than the cells from which they are derived (6). Thus, the overexpression of oligopeptide transporters may represent a component of the altered gene expression seen in cancers and could provide a growth advantage by augmenting the uptake of nutrients. It is possible that some cancers might overexpress other nutrient uptake systems to provide such a growth advantage. These other uptake pathways might also offer a putative target for low molecular weight anti-cancer drug targeting. An intriguing aspect of the oligopeptide transporters, however, is that these proteins are capable of shuttling substrates of molecular weight up to 350–400 Daltons (7). This is larger than transport systems which shuttle in nucleic acids or sugars, for example. Further, oligopeptide transporters are not very selective in their substrates (8). This promiscuous nature, along with its substrate size capabilities should improve the chances of finding anti-cancer agents which could act as oligopeptide transporter substrates.

Second, it is not clear which oligopeptide transporter might be the potential target in specific cancers. Several oligopeptide transporters have now been identified and new, iterative methods of searching the databases for such transporters have been described (9). Two prominent oligopeptide transporters have been described so far, PepT1 and PepT2, which have fairly similar substrate specificity. The RNA message for PepT1 is observed primarily in the small intestine and liver with lesser

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levels detected in kidney and pancreas (10,11). At present the intestine appears to be the primary site of PepT1 function in oligopeptide uptake. There is no evidence of oligopeptide uptake into the liver through PepT1 and it is not know whether the pancreas actually expresses functional PepT1 (10,11). PepT2 mRNA is primarily observed in the kidney and this protein is expressed functionally at high levels (12). A recent study has demonstrated another oligopeptide transporter in brain (13). All three of these proteins are structurally similar and it is likely that several other proteins of this class will be identified in other tissues. However, since we know so little about the putative expression of these different oligopeptide transporters in various cancer types, it may be necessary to exhaustively screen a variety of cancers for peptide transport function.

In this day of automation, miniaturization and high-throughput, an otherwise long and tedious functional screen is possible. There might be a rationale for focusing, and thus accelerating, this screen, however, by evaluating cancer cells for expression of a specific oligopeptide transporter protein. Recall the two observations made so far: a fibrosarcoma expressing a transporter which does not completely match the transport characteristics of either PepT1 or PepT2 and two pancreatic carcinomas which clearly express PepT1 predominantly. The majority of pancreatic carcinomas are derived from pancreatic duct epithelia and this tissue is derived from the same primitive gut tube that elaborates to produce the small intestinal epithelia where the PepT1 is normally expressed at high levels (10,11). Although it is difficult to establish general rules about cancer cells, even when they are derived from the same organ, it is possible that the gene expression patterns of a cancer may be related to the embryological derivation of that cell type. Thus cancer cells derived from tissues with an embryological heritage of the primitive gut tube, such as the exocrine pancreas, may express PepT1 preferentially over other oligopeptide transporters. The cell lines Caco-2 and HT-29 which are frequently used in cell culture to assess intestinal peptide transport were derived from colonic adenocarcinomas. The colon is not a site of significant PepT1 expression (10,11), but these colon cancer cell lines express levels of PepT1 comparable to small intestinal tissue. Fibroblasts, and thus the fibrosarcoma described to express a peptide transporter which does not behave as either PepT1 or PepT2, may be derived from a particular mesodermal component which could also be exploited similarly in a screening process.

Third, it may be useful to understand what drives the unanticipated expression of oligopeptide transporters in cancer cells. Just as there are a number of different cancer forms which can be derived from each tissue or organ, the driver behind each cancer form can also be varied (14). It is possible that a specific driver for each cancer could dictate the type or level of oligopeptide transporter expression. Although the expression of PepT1 would not be totally unexpected in normal pancreas, the overexpression of PepT1 in pancreatic cancer cell lines is surprising (2). Interestingly, both of the pancreatic cancer cells overexpressing PepT1 have an activated mutant form of K-*ras*. Although most pancreatic cancers do contain mutant K-*ras*, there is currently insufficient data to presume a connection between *ras*-driven cancer cell activation and oligopeptide transporter expression. Indeed, while the colon cancer cell lines HT-29 and Caco-2 both express PepT1 only HT-29 cells have been documented to carry a mutant K-*ras*. Interestingly, the fibrosarcoma cell line which expressed a non-PepT1, non-PepT2 oligopeptide transporter (1) carries a mutant N-*ras* allele (15). Although stimulation of *ras*-dependent pathways have been demonstrated to be a governor of gene expression (16), other pathways are activated in cancers which could act as a driver for oligopeptide transporter expression. Although no direct data supports any correlation between ras-driven cancer activation and any oligopeptide transport, such putative correlations may be a fruitful area of future study.

Fourth, we do not know what might limit the use of oligopeptide transporters as targets for cancer cells. If indeed there are specific drivers of cancers which stimulate the expression of these transporters, it is possible that other gene products driven in these cells may limit or restrict the effectiveness of anti-cancer agents (17). For example, it would be important to know if the expression of an oligopeptide transport in a cancer cell coincides with an upregulation of an efflux pathways (e.g. p-glycoprotein, multidrug resistance gene product). We do know that cancer cells have modified surface components and commonly lose competent contact interactions with adjacent cells or extracellular matrix (ECM) proteins. Ordinarily this loss of contact leads to a particular form of apoptosis known as anoikis (18). Cancer cells, however, fail to undergo apoptosis and continue to grow and spread after the loss of ECM interactions (19). Since interactions with ECM components control the polarity of epithelial cells and the differentiation of cells (20), the function of oligopeptide transporters in non-polarized and rapidly dividing cancer cells may be so variable or erratic such that it introduces another level of uncertainty for the approach of targeting to these transporters.

Established proton gradients at the surfaces of cells facilitate the uptake of substrates through oligopeptide transporters (21,22). The proton gradients established by epithelial cells in the small intestine and kidney utilize the polarized nature of these cells to maintain a pH differential at a particular membrane surface. Cancer cells typically lose this polarized phenotype and thus can not maintain the same type of transepithelial pH gradient to facilitate uptake through oligopeptide transporters. A systemic injection, rather than an oral delivery, of an anti-cancer agent capable of entering cells through an oligopeptide transporter substrate would circumvent unwanted uptake by PepT1 expressed at the apical plasma membrane of small intestinal epithelial cells. This compound, however, could still be taken up in a pH-driven fashion by PepT1 in non-cancer cells and by PepT2 in the kidney. It would seem unlikely that cancer cells, lacking a proton gradient, could effectively compete with these other cells for the substrate. However, we do know that pancreatic cancer and fibrosarcoma cells do absorb peptides quite efficiently *in vitro* and that the PepT1 expressed in pancreatic cancer cells was shown to reside substantially in endosomal-like vesicles (2). Future studies will need to address the nature of these vesicles. If they are acidic then cancer cells may compete very effectively for systemically administered oligopeptide transporter substrates. This may represent a function similar to that presumed for PepT1 in the liver which does not seem to absorb oligopeptides but may facilitate the efflux of oligopeptides generated in lysosomes into the cytoplasm.

In summary, it appears that we have either observed two rogue findings in the function of cancer cells or we are seeing for the first time a new arena of cancer cell targeting opportunities as it begins to be unveiled. If the overexpression of these

transporters in cancer cells turn out to be widespread, the potential to exploit these transporters in the targeted delivery of anticancer therapeutics would appear promising. Hopefully, the near future will provide the physiological and biochemical information required to clarify this putative pharmaceutical application of peptide transporters.

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