Folate Receptor-Mediated Drug Targeting: A Possible Strategy for Nonfunctioning Pituitary Adenomas?

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Clinically nonfunctioning pituitary adenomas (NFPAs) mostly arise from gonadotroph cells and account for about one-third of all pituitary tumors. Because they are not associated with hormone hypersecretion, these tumors are often diagnosed late when they are large and locally invasive. Consequently, the complete surgical removal of NFPAs is hard to achieve and they tend to recur. Medical treatment of NFPAs is still challenging because they are usually resistant to mainstay peptide receptor therapy with somatostatin (SRIF) analogs (octreotide and lanreotide) or dopamine agonists (bromocriptine and cabergoline). Indeed, although these agents may be useful in some cases to prevent residual tumor growth (1), their efficacy has not been confirmed in larger studies. Recently, SOM230 (pasireotide), a broad-range SRIF analog with affinity for SRIF receptor subtypes 1 to 3 and 5 (2) and BIM-23A760 (dopastatin), a chimeric agent interacting with both SRIF receptor and dopamine receptors were developed (3). However, NFPAs only partially respond to these compounds, at least in vitro (4, 5). After the discovery that NFPAs display overactivation of phosphatidylinositol 3-kinase/protein kinase B (AKT)/mammalian target of rapamycin pathway (6, 7), compounds inhibiting this signaling cascade at various levels have been tested against these tumors. The results are very promising, especially for dual inhibitors such as BEZ235 and XL765, which inhibit both mammalian target of rapamycin and the upstream phosphatidylinositol 3-kinase and exert a potent antiproliferative activity against primary human NFPA cells (8), rodent pituitary cell lines (9), and primary rat gonadotroph adenoma cells in vitro (10). In vivo studies are now required to prove or disprove the efficacy of these agents.

Conventional chemotherapy has been one of the major medical advances of the last decades, but it is usually associated with poor specificity for the cancer cells and high toxicity to the normal cells. To overcome these limitations, it would be ideal to identify ways to deliver a biologically effective concentration of anticancer agents to the tumor tissues with very high specificity. To reach this ultimate goal, extensive efforts have been undertaken to develop tumor-selective drugs by conjugating therapeutic agents to hormones, antibodies, and vitamin derivatives. Among them, folic acid holds great promise as a tumor-homing agent (11). Folate (or folic acid) is an essential B vitamin, which plays a pivotal role in cell survival by participating in the biosynthesis of nucleic and amino acids. Folate is internalized into the cells via a low-affinity reduced folate carrier protein or via high-affinity folate receptors. The best studied isoform of these receptors is folate receptor-α (FRα), a cell surface glycosyl phosphatidylinositol-anchored glycoprotein that can internalize bound folates and folate-conjugated compounds via receptor-mediated endocytosis (12).

Whereas FRα has a very restricted expression pattern in normal tissues, it is expressed at a high level in various human cancers, particularly epithelial carcinomas, including nonmucinous ovarian carcinoma, cervical carcinomas, and testicular choriocarcinomas, and less frequently in breast, endometrial colon, and renal cell carcinomas (13, 14). Due to its selective expression in tumor but not normal cells, FRα has become one of the most investigated cellular surface antigens for targeted delivery of a variety of molecules, including imaging agents, chemotherapeutic agents, oligodeoxynucleotides, and macromolecules. Various types of drug carriers have been conjugated to folate such as liposomes, lipid nanoparticles, polymeric nanoparticles, polymers, and micelles filled with the molecule that needs to be delivered (11). FRα-targeted liposomal

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Abbreviations: FRα, folate receptor-α; NFPA, nonfunctioning pituitary adenoma; SRIF, somatostatin.

doxorubicin has been evaluated for its antitumor potential against FRα-positive solid tumors in both in vitro and in vivo (xenografts) models with success (15).

In the current issue of *Endocrinology*, Liu and colleagues (16) report on a new potential therapeutic approach for NFPAs that takes advantage of the expression of FRα in these tumors. Gene expression array analysis of pituitary adenomas had previously shown that the *FOLR1* gene, encoding FRα, is overexpressed in NFPAs but not in hormone-secreting adenomas (17, 18), and consequently, FRα-positive pituitary tumors could be detected by [^{99m}Tc]folate single-photon emission computed tomography/computed tomography imaging in vivo (19). It was recently confirmed by the laboratory of Rhenzi Wang (20) that most NFPAs (74%) indeed express FRα on their tumor cell membrane, as determined by immunohistochemistry. In contrast, the receptor was not detected in functioning adenomas, nor was it detected in normal pituitaries. Moreover, the expression of FRα was significantly more abundant in invasive NFPAs when compared with noninvasive tumors, and it correlated with the proliferation rate of the tumors (Ki67 labeling index) (20). These results are in agreement with previous findings reporting that ectopic overexpression of FRα in gonadotroph cells enhances cell growth (21).

In the featured article, the authors exploit the presence of FRα on pituitary tumor cells to deliver the cytotoxic drug doxorubicin to human primary NFPA cells grown as 3-dimensional culture microtissues, more representative of the in vivo situation than classical 2-dimensional cultures. They show that the incubation with FRα-targeted liposomes loaded with doxorubicin decreases the viability of FRα-positive tumor cultures but not that of FRα-negative cultures. This effect on cell survival is due to the induction of apoptosis through the activation of caspase-8, caspase-9, and caspase-3/7. Interestingly, the authors could also show that doxorubicin suppresses the capacity of FRα-positive NFPA cells to invade through Matrigel. This phenomenon is likely due to a doxorubicin-mediated down-regulation of matrix metalloprotease-2 and -9 expression, two enzymes responsible for the proteolytic processing of extracellular matrix structural proteins and thereby able to regulate cell migration. Liposomes filled with doxorubicin, but unconjugated to folate, affect viability and invasion of NFPA cells to a much lower degree and show similar unspecific effects on all cultures tested, regardless of the cells being FRα-positive or -negative. Due to the lack of FRα expression in normal adenohypophyseal cells, it can be hypothesized that a targeted delivery of doxorubicin would offer the high specificity and low toxic side effects expected from an effective antitumor therapy.

In the context of pituitary adenomas, chemotherapy is used mainly as salvage therapy in aggressive cases or for pituitary carcinomas, but the outcome has been often unsatisfactory (22). Radiotherapy is also an option for lowering the risk of recurrence, but because it may be the cause of high morbidity due to brain necrosis, radiation damage to the optic pathways, or hypopituitarism, it is often used only in selected cases. Doxorubicin has not been previously employed as a chemotherapeutic drug against pituitary adenomas, although it is extensively used against a variety of solid tumors including bladder, breast, stomach, lung, ovaries, and thyroid as well as against hematological malignancies. Temozolomide, an oral alkylating agent, has recently been used to treat aggressive pituitary adenomas with promising results. In a recent article, Raverot and colleagues (23) review the studies reporting the successful use of temozolomide in the management of advanced pituitary adenomas or carcinomas and report that tumor response (defined as 20% decrease in maximal tumor size) was noted in 38% of treated NFPA patients and was similar to the response rate of GH-secreting adenomas (33%) but was lower than that of ACTH-secreting (56%) or prolactin-secreting (66%) adenomas. Therapy response was usually dependent on the level of expression of *O*(6)-methylguanine-DNA methyltransferase, a DNA repair enzyme that potentially interferes with drug efficacy. Moreover, initial response to temozolomide was not always predictive of short- or long-term tumor control (23). Therefore, there is still room for improvement in the therapy of aggressive NFPAs.

The findings by Liu and colleagues (16) that doxorubicin inhibits the viability of pituitary tumor cells and also hinders their capacity to invade propose a novel treatment strategy that might be highly beneficial for the management of patients with aggressive NFPAs.

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