The Epidermal Growth Factor Variant III Peptide Vaccine for Treatment of Malignant Gliomas

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KEYWORDS

- Epidermal growth factor receptor variant III
- Epidermal growth factor receptor • Cancer vaccine
- Immunotherapy • Glioblastoma multiforme

MALIGNANT GLIOMAS AND PEPTIDE VACCINATION

There have been significant advances in the understanding of the molecular and cellular biology of malignant gliomas in recent years. Unfortunately, the median survival of patients who have newly diagnosed glioblastoma multiforme (GBM) still remains 14.6 months after surgery followed by postoperative radiation therapy and temozolomide.[1] Despite several different therapeutic approaches, at the clinical and preclinical stage, the median overall survival of these patients has not changed significantly and current standard of care treatment results in toxic side effects to normal brain and hematopoietic function. One of the main difficulties in the treatment of this tumor is its highly invasive and diffuse spread through the brain parenchyma. Under these circumstances local therapies are ineffective and systemic therapies are limited by the blood brain barrier and lack of specificity. New systemic therapies are needed and unique approaches from immunotherapy have emerged over the past two decades with promising results. Immunotherapy has the advantage of being tumor specific and systemic in delivery. Examples of immunotherapeutic approaches include treatment with tumor-specific monoclonal antibodies (MAb) alone, MAb attached to cytotoxic agents, or vaccination with tumor-associated antigens. One of the main challenges to immunotherapy, however, has been finding the ideal tumor associated antigen and overcoming the tumor's ability to downregulate the surrounding immune microenvironment. In this article, the authors discuss a peptide vaccination strategy developed around the tumor-associated antigen epidermal growth factor variant III (EGFRvIII).

Peptide vaccines are chemically synthesized based on the sequence of tumor-associated antigens and are injected subcutaneously or intravenously into patients who have cancer to stimulate a tumor-specific immune response. This delivery method is safe, inexpensive, and the peptide itself is easy to produce and store. Peptide vaccination strategies for cancer therapy have been studied since the early 1990s with the identification of MAGE-1, the first human cancer associated gene, which encodes melanoma-specific antigens.[2] That discovery was followed by the description of the first human tumor-specific peptide, which was nine amino acids long and restricted by HLA-A1.[3] Since then there have been many clinical trials using peptide-based vaccines for the treatment of a variety of cancer types. The exact mechanism of immune activation

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after peptide vaccination is not known, but theoretically the peptides are processed by antigen-presenting cells and presented to T cells in the context of major histocompatibility complex (MHC) molecules.

EPIDERMAL GROWTH FACTOR VARIANT III

The epidermal growth factor (EGF) receptor is a transmembrane tyrosine kinase growth factor receptor that belongs to a family of four related receptors: EGF receptor (ErbB-1), ErbB2/Neu/Her2, ErbB3/Her3, and ErbB4/Her4.[4] Ligand binding to the monomeric receptor leads to dimerization, resulting in activation of cytoplasmic catalytic function and subsequent autophosphorylation.[5] Following autophosphorylation, numerous intracellular signaling pathways are activated with a multitude of downstream effects ranging from cell division and migration to adhesion, differentiation, and apoptosis.[6] Aspects of EGF-receptor signaling have been implicated in the pathogenesis of many human cancers as many tumor types exhibit over expression or aberrant activity of the EGF receptor[7,8] leading to unregulated growth and malignant transformation. Cetuximab and panitumumab, two monoclonal antibodies against the EGF receptor, and erlotinib and gefitinib, two EGF receptor tyrosine kinase small molecule inhibitors, are examples of EGF receptor antagonists that have been approved by the US Food and Drug Administration. They are currently available for treatment of metastatic non-small cell lung cancer, squamous cell carcinoma of the head and neck, colorectal cancer, and pancreatic cancer[9] and are now being investigated in many clinical trials for a variety of tumor types including malignant gliomas. However, EGF receptors have wide expression in normal tissues and therapeutics directed against it theoretically could lead to unwanted toxic effects. An antigen present only in tumor tissue would be a more ideal target.

There are at least ten classes of EGF receptor genomic variants described in gliomas. EGFRvIII is the most common variant of the EGF receptor and is present in 24% to 67% of GBM where it was first identified,[10,11] but has not been observed in normal brain. This mutant receptor has subsequently been detected in many other solid tumors including medulloblastomas, breast, colon, ovarian, metastatic prostate, head and neck, and non-small cell lung carcinomas.[12–18] There is debate over the prognostic significance of this mutant receptor in malignant gliomas, but the most recent and largest study indicated that EGFRvIII is a negative prognostic indicator when considering long-term GBM survivors only.[19–21]

EGFRvIII results from an in-frame deletion corresponding to exons 2 to 7 of the EGF receptor gene resulting in the fusion of exon 1 to exon 8 and deletion of a large portion of the extracellular domain (**Fig. 1**). This deletion removes amino acids 6 to 273 and generates a novel glycine at the junction. This novel epitope is at the amino terminus of the extracellular domain of the truncated receptor resulting in a protein of 145 kDa compared with the wild-type EGF receptor, which is 170 kDa. Although it does not bind ligand, EGFRvIII is constitutively active and can lead directly to cancer phenotypes because of its oncogenic properties.[22] The molecular mechanism by which this mutant receptor acts is not completely known. Constitutive activity of phosphatidylinositol 3 kinase and c-Jun N-terminal kinase have been implicated.[23,24] EGFRvIII is an attractive target for malignant gliomas because it is not expressed in the normal brain and because cells producing EGFRvIII have an enhanced capacity for unregulated growth, survival, invasion, and angiogenesis. It is an especially valuable target for immunotherapeutic approaches because the juxtaposition of ordinarily distant amino acids plus the unique glycine at the junction produces a highly immunogenic and novel epitope. Tumor antigens are generally over-expressed self-antigens that have triggered immune tolerance. Peptide vaccination with these tumor antigens carries the risk of either an autoimmune response or a muted immune

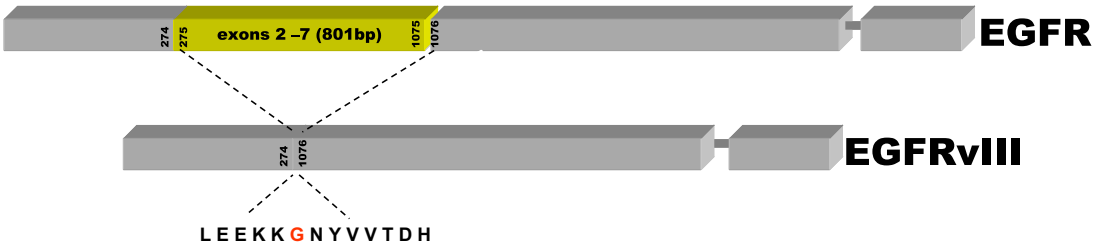

Fig. 1. EGFRvIII is an alternatively spliced form of the EGF receptor that is the result of the deletion of exons 2 to 7 removing 801 base pairs from the extracellular domain of the receptor. The fusion on exons 1 to 8 results in a novel glycine.

response because of tolerance. EGFRvIII is seen only on tumor cells and therefore has a less likely risk of autoimmunity or muted response because of immune tolerance.

PRECLINICAL TRIALS STUDYING ACTIVE IMMUNIZATION WITH EPIDERMAL GROWTH FACTOR VARIANT III

The unique extracellular peptide sequence of the EGFRvIII junction can be used to raise polyclonal and monoclonal antibodies that specifically recognize EGFRvIII[10,25] but not wild-type EGF receptor. The ability of this peptide to elicit a humoral response in rabbits suggested that a vaccine based on this novel antigen could be used against tumors, although evidence of a T-cell reaction was also needed for full antitumor response. Moreover, Purev and colleagues[16] evaluated the serum of subjects who had EGFRvIII-expressing breast cancer and found EGFRvIII-specific antibodies and an EGFRvIII-specific lymphoproliferative response suggesting that patients may benefit from vaccination against EGFRvIII by boosting existing immune responses.

Moscatello and colleagues[26] were the first to demonstrate the possibility of using a peptide derived from EGFRvIII as an antitumor vaccine in mouse models. For this study, the 14 amino acid peptide corresponding to the exon 1 to exon 8 junction (LEEKKGNYVVTDHC, where the terminal cysteine was added for conjugation) was conjugated to keyhole limpet hemocyanin (KLH) and used as the peptide vaccine. KLH is a well-established carrier protein for conjugated peptide vaccines that increase peptide half-life, provides strong CD4 T helper response, and activates antigen-presenting cells.[27] NIH Swiss mice or Fischer 344 rats were pre-immunized with peptide-KLH conjugate and subsequently injected subcutaneously with NIH3T3 cells transformed by the overexpression of human EGFRvIII. Pre-immunization with this peptide vaccine significantly decreased tumor incidence when compared with controls, and the antitumor effect was long lasting as seven animals that had successfully rejected the original tumor were rechallenged 6 to 12 months later and showed no tumor growth. This peptide conjugate was also able to mediate the rejection of established tumors in mice, a paradigm for the treatment of human disease. The magnitude of the regression was significant where tumors of up to 4 cm^3 completely involuted within 3 weeks. In a few of the mice treated, a secondary tumor recurred approximately 40 to 50 days later. Upon analysis, four of five tumors no longer expressed EGFRvIII demonstrating that the vaccine treatment successfully targeted cells expressing the antigen. Immunologic studies revealed that the tumor regression was dependent on an EGFRvIII-specific CD8+ T lymphocyte mediated cytotoxic response that did not recognize cells expressing wild-type EGF receptor.

In a subsequent study, mice pre-immunized with the identical peptide vaccine as the prior study, which they called pep-3-KLH,[28] were challenged subcutaneously or intracerebrally with murine melanoma cells expressing artificially generated mouse EGFRvIII. A significant antitumor effect was also noticed in the pre-immunized mice. They noted a significant humoral response in these vaccinated mice, but not a cytotoxic T-cell response. Passive transfer of sera from immunized mice to non-immunized mice protected against tumor development suggesting that antibodies were responsible for the response. Depletion studies showed that the CD8+ T cells and natural killer cells were important for the antitumor effect, and in vitro assays showed that macrophages could lyse target tumor cells with serum from the pep-3–KLH–vaccinated mice raising speculation that these were effector cells for antibodies. Another group of mice that had been injected intracerebrally were also vaccinated after tumor inoculation. Mice with established intracerebral tumors injected with the pep-3-KLH exhibited a 26% increase in survival. As had been seen by Moscatello and colleagues, 80% of recurrent tumors in this study no longer expressed EGFRvIII.

In a variation to the vaccine, Ciesielski and colleagues[29] created a homo-chimeric peptide vaccine with multiple copies of the EGFRvIII epitope linked together by a lysine bridge (MAP). In rats pre-immunized with MAP, the authors demonstrated attenuated tumorigenesis of EGFRvIII expressing cells but not wild-type EGF-receptor expressing tumor cells. Moreover, the median survival of pre-immunized rats was increased 72% more than unvaccinated controls injected with intracerebral EGFRvIII expressing tumor cells. Supplementation with granulocyte-macrophage colony-stimulating factor (GM-CSF) led to increased titer of EGFRvIII-specific antibodies and increased recruitment of CD4+ and CD8+ T cells at the tumor site. Splenocytes and CD8+ T cells from vaccinated rats produced IFN-γ in vitro in response to stimulation by rat glioma cells expressing EGFRvIII, but not by those expressing wild-type EGF receptor, suggesting a role for cell mediated immunity in the antitumor response.

Heimberger and colleagues attempted an additional method of EGFRvIII antigen presentation by way of dendritic cells (DC). Mice pre-immunized

with DC mixed with pep-3-KLH and then intracerebrally challenged with melanoma cells expressing EGFRvIII had an approximately 600% increase in median survival time compared with unvaccinated controls.[30] Pre-immunized mice that survived tumor challenge were reinjected 100 days later in the contralateral hemisphere and survived showing long-lasting protection, confirming the results of Moscatello and colleagues.[26]

Using a bioinformatic and combinatorial peptide approach to find a peptide that could evoke superior immune response, Wu and colleagues[31] screened the EGFRvIII peptide sequence with two software programs to predict candidate epitopes restricted by the MHC class 1 subtype HLA-A0201, which is the predominant subtype in most ethnic groups. Three peptides were predicted, synthesized, and then subsequently loaded into mature human DC from peripheral blood monocytes. Autologous CD8+ T cells were then stimulated in vitro with DC mixed with the peptides. As demonstrated by IFNγ production and cytotoxicity against HLA-A0201+ EGFRvIII transfected U87 glioma cells, one of the three peptides was found to induce an EGFRvIII-specific cytotoxic T lymphocyte response. The difference between peptide one (LEEKKGNYV), which was the successful peptide, and peptide two (LEEKKGNYVV) was only one amino acid.

CLINICAL TRIALS STUDYING ACTIVE IMMUNIZATION WITH EPIDERMAL GROWTH FACTOR VARIANT III

Encouraging results from these preclinical studies have resulted in clinical trials evaluating active immunization with the EGFRvIII peptide as defined by Moscatello and colleagues.[26] The first Phase I trial was performed by the Southwestern Oncology Group (protocol S0114)[32] for subjects who had prostate and ovarian cancer. The study evaluated the safety of vaccinating patients who have the EGFRvIII peptide administered with KLH and GM-CSF intradermally once every month for 6 months. There were no serious adverse events among the 10 subjects who enrolled. The next trial was Vaccine for Intra-Cranial Tumors I, a Phase I clinical trial for subjects who had newly diagnosed malignant gliomas (World Health Organization grade III or IV) to determine the safety of vaccinating with mature DC loaded with the 500 μg of vaccine conjugate. Twenty subjects were enrolled, but four of these subjects did not qualify for the vaccine. Beginning 2 weeks after surgical resection and postoperative radiotherapy, subjects were vaccinated 2 weeks apart for a total of three vaccinations. There was a lack of delayed-type hypersensitivity reaction to KLH or the EGFRvIII peptide before vaccination, but after vaccination 16 out of 16 subjects (3 grade III, 13 GBM) reacted to KLH, and 10 out of 16 (62.5%) reacted to the peptide. There was a detectable humoral response although all subjects tested had significantly higher antigen-specific T-cell proliferation in vitro after vaccination in response to peptide and KLH. Of the two subjects who did not undergo gross total resection, one subject who had glioblastoma presently remains alive 6.2 years later and had a complete response after vaccination, whereas the other subject who had anaplastic astrocytoma is alive and progression-free 5.4 years later. Two of the three subjects who had grade III tumors are alive and with evidence of recurrence at 66.2 and 123.7 months after vaccination, whereas one subject recurred 47.7 months after vaccination. For subjects who had GBM, the median survival time was 775.6 days, significantly higher compared with published trials evaluating subjects who were newly diagnosed with GBM.[33,34]

A third clinical trial, A Complementary Trial of an Immunotherapy Vaccine Against Tumor-specific EGFRvIII (ACTIVATE), was a Phase 2 trial that evaluated the efficacy of the peptide vaccine conjugate for 23 subjects who had newly diagnosed EGFRvIII-expressing GBM as assayed by immunohistochemistry or polymerase chain reaction. To be included, the subjects must have undergone gross total resection followed by conformal radiation and concurrent temozolomide (75 mg/m^2/d) but not have shown tumor recurrence. Intradermal vaccine inoculation was done concurrently with GM-CSF in 2-week intervals for three doses followed by monthly injections until tumor progression.[34–36] The vaccination resulted in an EGFRvIII-specific humoral response with minimal toxicity. Using an immunoassay developed by Schmittling and colleagues,[37] the authors were able to detect increased levels of anti-EGFRvIII and anti-KLH antibodies in the serum of subjects in this trial after vaccination. Moreover, there was an induction of CD8+ IFNγ EGFRvIII-specific T cells as a result of vaccination. The median time to progression was 64.5 weeks and the median survival was 126.1 weeks. Historically matched controls had a median time to progression of 28.5 weeks and median survival of 56 weeks. Similar to the preclinical studies, recurrent tumors from subjects after vaccination no longer expressed EGFRvIII.

Although the ACTIVATE study was ongoing, results were published from a randomized trial in subjects who had newly diagnosed GBM, which showed radiotherapy plus continuous daily temozolomide[1] followed by six cycles of adjuvant

temozolomide demonstrated prolonged median survival of 14.6 months when compared with 12.1 months for subjects receiving radiotherapy alone. Because the standard of care for newly diagnosed patients who have GBM has now become radiotherapy with concurrent temozolomide followed by six cycles of adjuvant temozolomide, the ACTIVATE II trial was initiated with 21 subjects who were newly diagnosed with GBM and underwent gross total resection followed by concurrent radiotherapy and temozolomide. Currently, the vaccine for this and subsequent trials is now produced by Celldex Therapeutics and is called CDX-110. CDX-110 vaccination is given on day 21 of the 28-day temozolomide cycle when the immune system has theoretically recovered from the temozolomide-induced immune suppression. Many chemotherapeutic agents, including temozolomide, induce pronounced lymphopenia that prevents an effective combined immunotherapeutic approach. However, chemotherapy can be integrated well with tumor vaccines by taking advantage of their pharmacodynamic properties.[38] Results from this trial demonstrated no diminution of the EGFRvIII-specific immune response after co-administration of temozolomide with EGFRvIII vaccination. Although the trial has not reached final median survival, the preliminary data reveals median time to progression of 16.6 months and median overall survival of 33.1 months when compared with historical matched controls that had a median time to progression of 6.4 months and median survival of 14.3 months.[36,39]

Currently, patients who are newly diagnosed with EGFRvIII-positive glioblastoma are being enrolled into ACT III, a phase II/III randomized multicenter nationwide clinical trial sponsored by Celldex Therapeutics. Subjects were randomized 2:1 into a treatment group including gross total resection followed by radiation, chemotherapy and CDX-110 vaccination, or the control group receiving gross total resection followed by radiation and chemotherapy without vaccination. A recent amendment to the trial will enroll all eligible subjects into the vaccine arm.[40]

DISCUSSION

Among the many tumor-specific mutations found in brain tumors, those with unique extracellular epitopes are the most useful practically for therapeutic purposes. EGFRvIII is an ideal target antigen for immunotherapy because it is a unique epitope that is expressed only in cancer cells. Several preclinical studies have focused on understanding and the targeting of this mutant EGF receptor. Therapeutic approaches have included the use of unarmed MAb, immunoliposomes, radiolabelled MAb, MAb conjugated to immunotoxins or boronated dendrimers, and small molecule inhibitors. However, active immunization with the peptide vaccine is the only strategy that has shown the most promise and has successfully made it to clinical trials. The use of EGFRvIII extracellular peptide sequence elicits specific immune response. Data from early clinical trials have been extremely promising, especially given the poor prognosis of patients who express EGFRvIII.

The ongoing, randomized clinical trial evaluating CDX-110 is highly pivotal. If successful, this will be the first randomized trial demonstrating the efficacy of CDX-110 and the first trial demonstrating a successful cancer peptide vaccine. Should the vaccine be effective in prolonging overall survival in patients who have newly diagnosed GBM, the vaccine could be extended to other tumor types that express this target. As discussed earlier, the two largest challenges to immunotherapy are finding the appropriate target antigen and overcoming the local tumor immune suppression. GBM downregulate or express defective HLA antigens[41] and alter MHC class II expression of nearby microglia and macrophages.[42] The overall number of T cells is decreased in patients who have GBM with a decrease of effector T cells and an increase in the T-regulatory cells.[43] Combining EGFRvIII targeted therapies with an immune modulator may help the immune system to mount a larger response against the tumor. One such immune modulator was studied by Hussain and colleagues[44] who showed that signal transducers and activators of transcription 3 blockade by a novel small molecule can reverse immune tolerance in patients who have GBM.

Malignant neoplasms are complex and have a heterogeneous group of genetic alterations. A single targeted approach is unlikely to cure patients. As seen in the preclinical trials and the early clinical trials with the EGFRvIII vaccine, recurrences occur even after successful vaccination. The tumors that recur are no longer expressing EGFRvIII, suggesting that recurrent tumors find an alternative mechanism of tumorigenesis. Trials involving a combinatorial approach, such as that proposed by Huang and colleagues,[45] will likely be evaluated in future studies. Targeting of multiple pathways may prove to be more effective than targeting a single antigen. With advancing technologies and increasing knowledge, the hope is that we will one day be able to resect a tumor, analyze the tumor, and treat patients with targeted therapies directed specifically to the biology of that specific tumor.

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