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OSU-03012 in the Treatment of Glioblastoma

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Running Title: OSU-03012 and Glioblastoma

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ABSTRACT

An article presented in this issue of *Molecular Pharmacology,* by Yacoub *et al.,* examines the actions of OSU-03012 on both primary and glioblastoma cell lines. The author's found that OSU-03012 could induce tumor cell death by itself but also acted as a strong sensitizing agent to radiotherapy-induced cell death. Glioblastoma cells were also more sensitive to this compound than non-transformed astrocytes. Radiation-induced cell death was refractory to siRNA-directed inhibition of PDK1 but not OSU-03012. These results indicate that OSU-03012, which has been thought to primarily mediate anti-tumor effects via the inhibition of PDK1, has actions independent of PDK1. Furthermore, the authors demonstrated that the effects of OSU-03012 were independent of ERB-B1-vIII and PTEN expression. These are important findings as they start to identify a new mechanism to sensitize glioblastoma cells and also suggest that OSU-03012 could be combined with existing inhibitors to further sensitize tumor cells. In glioblastoma cells, OSU-03012 appeared to induce apoptosis via ER stress-induced PERK-dependent signaling. OSU-03012-induced death of the glioblastoma was only weakly suppressed by the pan-caspase inhibitor, zVAD, suggesting that OSU-03012-induced cell death was largely caspase independent. Overall, these are exciting results and suggest new more effective treatment options may be obtainable for people suffering from these deadly tumors.

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Glioblastomas are the most common tumor arising from the CNS. Approximately 74,000 glioblastomas are diagnosed worldwide each year (1) and there are indications that the incidence of these types of tumors is increasing (2). Glioblastoma multiforme is a malignant neoplasm that accounts for approximately 55% of all gliomas (3). Glioblastoma multiforme tumors, arise from astrocytes, usually occur in the cerebellum, are irregularly shaped, and contain focal areas of necrosis.

If the glioblastoma is resectable, surgery is typically performed. However, there is some question as to the benefits of the surgical approach (4). Radiotherapy following surgical removal of the tumor has been shown to have some benefit (4). For those tumors that are not resectable, radiotherapy is often used as the primary form of treatment (4). However, even with radiotherapy the prognosis of patients having certain gliomas can be quite poor (5). Despite all the advances that have been made in cancer treatment; the proportion of individuals that die from glioblastomas multiforme is remarkable. Less than 4% of individuals survive for more than two years with glioblastoma multiforme, the most common glioblastoma (6). Close to 60% of individuals with this type of cancer die within 6 months (6).

The combination of chemotherapy and radiotherapy does not appear to produce additive or synergistic effects in regards to the treatment of gliomas (7, 8). Part of overall difficulty in treating these tumors is that they can be very complex and heterogeneous from one another. Glioblastoma multiforme can develop as the first occurrence of cancer in the body or they can develop from lower grade gliomas (9). In addition, approximately 25% of glioblastomas express the mutant EGFR receptor ERB-B1 vIII (3). Tumors from glioblastoma patients may or may not express proteins such as p53 or p16 ((4) and Yacoub *et al.* this issue). Treatment alternatives which could preferentially target glioblastoma cells and would be effective against a wide variety

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of glioblastomas would potentially benefit patients suffering from this deadly disease. The report by the laboratory of Paul Dent suggests that OSU-03012 has this potential.

With the discovery that many cellular signaling pathways can have a role in transformation and in the prevention of cell death; many efforts have been made to inhibit protein kinases within these pathways to prevent the growth of cancer cells or induce their demise. The components of the PI3K and ERK signaling pathways are especially attractive targets as they can mediate both proliferative and anti-apoptotic responses (10). A number of efforts are underway to examine the ability of both ERK and PI3K inhibitors to induce apoptosis of transformed cells (11). Probably the most successful approach to targeting cellular signaling pathways at this point in time has been with Gleevec (imatinib mesylate/ST1571) (12). Gleevec targets the Bcr-Abl kinase and has had good success in treating individuals with leukemia due to the Philadelphia chromosome, although some patients will develop resistance to this treatment (12). A part of the success of this treatment is due to the specificity of Gleevec for tumor cells. Additional efforts are being put forth to determine if inhibitors of the components of different signaling pathways can synergize with conventional treatments. There has also been success in this area of research as both ERK and PI3K inhibitors will synergize with radiotherapy and chemotherapy to induce cell death (12, 13).

OSU-03012 is a celecoxib derivative that reportedly inhibits PDK1 (14), an important kinase in signaling via the anti-apoptotic PI3K pathway. Because of this activity, there has been an interest in determining if this compound would be effective in inducing apoptosis by itself or with other cancer treatments, similar to the approach that has been used with other inhibitors of cellular signaling pathways. OSU-03012 has been shown to be effective in inducing cell death in a variety of tumors types; such as pancreatic (15), colon (16), breast (17), and glioblastoma (Yacoub *et al.* this issue). In addition, it would appear that this compound can act in synergy

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with Gleevec to induce tumor cell death in individuals that have developed resistance to Gleevec alone (18).

It is a rational idea that OSU-03012 mediates these effects through the inhibition of PDK1 (15). However, it is possible that OSU-03012 could also mediate additional anti-tumor effects independent of PDK1. Celecoxib the parent compound of OSU-0012 has been shown to induce apoptosis via ER stress and the induction of CHOP (19). Two recent abstracts from the American Association for Cancer Research meeting indicate that OSU-03012 can kill cells by multiple mechanisms and one of these abstracts showed a poor correlation between PDK1 inhibition and OSU-03012 lethality (20, 21) In the prototypical ER stress pathways PERK has been reported to modulate eIF2 alpha phosphorylation and the subsequent expression of CHOP (22). The manuscript from the laboratory of Paul Dent is the first to describe the ability of OSU-03012 to induce tumor cell killing via an ER stress and PERK-dependent pathway. In addition, at the low concentrations of OSU-03012 used by this group, detectable increases in the phosphorylation of eIF2 alpha were not observed suggesting that PERK was mediating apoptotic activities by another mechanism independent of CHOP. Figure 1 depicts the novel mechanism of action of OSU-03012-induced cell death in glioblastoma cells. These are novel results which will start to identify additional targets that can be used for the treatment of glioblastoma. In addition, the results also indicate that screening of OSU-03012 derivatives on the basis of their ability to inhibit PDK1 may not be the best approach to find novel compounds that could also be used for cancer treatment as OSU-03012 also induces cell death by a form of ER stress.

OSU-03012 triggers cell death independent of; BIM, BAX, or BAK; caspase activation; ERB-B1-vIII; p53; p16; and PTEN expression in glioblastoma cells. The data in Table 1 and Figure 2A of the Yacoub *et al.* publication in this issue of *Molecular Pharmacology* demonstrates that OSU-03012 could induce apoptosis in a number of glioblastomas that demonstrate a large amount of heterogeneity from each other. This is a good characteristic for a

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drug to have considering how heterogeneous different glioblastomas can be. In addition, the effects of OSU-03012 were more pronounced on the transformed cell lines and the primary glioblastoma cells than they were on primary astrocytes, indicating some selectivity of the treatment for tumor cells. One of the most exciting aspects of this work is the ability of OSU-03012 to synergize not only with an existing therapy for glioblastomas (radiotherapy) but also, because of its novel mechanism of action, to synergize with inhibitors of the cellular signaling pathways (Figure 2). Based on the separate mechanisms of action it will be important to determine if the combination of radiotherapy, OSU-03012, and a kinase inhibitor promotes glioblastoma cell death to an even greater extent.

In summary: Glioblastomas are a very deadly cancer and successful treatment protocols are lacking. The identification of new treatments or identification of mechanisms to make existing treatments more successful is needed. The investigations of Yacoub *et al.* indicate that OSU-03012 may be a compound that has promise in doing so. The next step will be to complete successful *in vivo* studies both using the compound by itself and in conjunction with different therapies.

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FIGURE LEGENDS

Fig. 1. Proposed pathway by which OSU-03012 leads to cellular apoptosis. OSU-03012 induces an ER stress response and the subsequent activation of PERK. PERK activation leads to the release of cathepsins from lysosomal compartments and the released cathepsins cleave Bid. Bid acts on the mitochondria were it causes the release of apoptosis inducing factor.

Fig. 2. OSU-03012 synergizes with radiotherapy and protein kinase inhibitors to increase cell death.