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Small-molecule inhibitors of p21 as novel therapeutics for chemotherapy-resistant kidney cancer

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Abstract

“In the search for novel approaches for the treatment of resistant cancers, small molecules targeting the cyclin dependent kinase inhibitors have shown considerable promise”

Keywords

p21; kidney cancer; apoptosis; small molecule inhibitor; chemo-resistance

Nearly 65,000 people were diagnosed with kidney cancer in the United States in 2012, of which 92% cases are with renal cell carcinoma (RCC). Over 13,500 patients are expected to die in 2012 of kidney cancer. Most importantly, RCC is one of the few cancers whose incidence is actually increasing (with a rate of about 2% every year). Approximately 30% of the RCC patients are diagnosed when the disease is already metastatic, at which time it has 95% mortality. Treatment options are limited for those patients since the metastatic kidney cancer is notoriously resistant to chemotherapy, hormone therapy, and most other available treatments. As a result, the average survival time of patients with metastatic kidney cancer is 12 – 18 months, and only 11.1% will survive for longer than 5 years. Standard chemotherapy with cytostatic agents may shrink the tumor temporarily, but drug resistance will eventually occur. More importantly, such treatments have all shown no benefit to survival. A possible reason for chemotherapy resistance is, when used alone, these agents

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#Equal contribution

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cause resistance to apoptosis, since inactivation of apoptosis is essential for cancer development [1]. In order to extend the lives of these patients, novel treatments to overcome such drug resistance are needed.

Angiogenesis is an attractive therapeutic target for cancer and anti-angiogenesis targeted therapy has been used for the treatment of kidney cancer. Anti-angiogenic drugs comprise a broad family of agents that target the tumor vasculature including small molecule inhibitors or monoclonal antibodies of growth factor receptors, vascular endothelial growth factor (VEGF), and multiple growth factor receptors. For clear cell kidney cancer, tyrosine kinase inhibitors (TKI) such as sunitinib (Sutent), pazopanib (Votrient), and sorafenib (Nexavar) have been approved for treatment of RCC. Axitinib (Inlyta) is another TKI and has been approved to treat advanced RCC. Sorafenib is a multi-kinase inhibitor taken orally and has been approved for use in the treatment of metastatic RCC. Bevacizumab (Avastin), an anti-angiogenic humanized monoclonal antibody against VEGF, when used with interferon alfa, has also been shown to slow tumor growth for people with metastatic RCC.

Although there is often a high initial responsiveness with anti-angiogenic drugs, at present, angiogenesis inhibitors have been shown to prolong progression-free survival but they exert only a limited effect on overall survival in cancer patients. In almost all treated patients tumors eventually become resistant to angiogenesis inhibitors. Therefore, new targeted therapy is urgently needed to combat drug-resistant cancer including kidney cancer.

“In light of the anti-apoptotic function of p21 in RCC, selective p21 inhibitors could be useful to attack therapy-resistant kidney cancer at their Achilles’ heel.”

An important area of research is to identify the mechanism of chemotherapy resistance in RCC, which is likely due to an exuberant DNA repair mechanism mediated by the p53 tumor suppressor pathway, and subsequent induction of the downstream anti-apoptotic molecule p21 (also known as p21^{WAF1/Cip1}) [2,3]. p21 is a cyclin-dependent kinase (CDK) inhibitor and has shown pleiotropic effects on cell growth and apoptosis in both malignant and non-malignant cells and tissues [2]. Based on the intracellular localization and the stage of differentiation, p21 protein executes various functions in the cell. When residing in the nucleus, p21 binds to and inhibits the activity of several cyclin dependent kinases and blocks the transition from G1 phase into S phase, or from G2 phase into mitosis after DNA damage. Therefore, p21 acts as a tumor suppressor. p21 is also an important protein for the induction of replication senescence as well as stress-induced premature senescence (SIPS) after DNA damage [4]. SIPS is a sustained growth-arrested state in which cells are alive and secrete factors which may promote cancer progression. In the cytoplasm, p21 protein prevents apoptosis and acts as an oncogene [5]. It is able to bind to and inhibit caspase-3, as well as the pro-apoptotic kinases apoptosis signal-regulating kinase 1 (ASK1) and Jun N-terminal kinase (JNK) [4]. The function of p21 in response to a DNA damage depends on the extent of the damage. In the case of low-level DNA damage, the expression of p21 is increased. It induces cell cycle arrest, and performs also anti-apoptotic activities. However, after extensive DNA damage the amount of p21 protein is decreased and the cell undergoes apoptosis [4]. This dual function of p21 was also observed in oncogenesis. A better understanding of the functional role of p21 in various cancer related conditions will assist in the development of better cancer treatment strategies.

Overexpression of cytoplasmic p21 is found in a variety of human cancers including RCC, breast cancer, pancreatic cancer, testicular cancer, ovarian cancer, cervical cancer, squamous cell carcinomas and prostate cancer. In many cases, p21 upregulation correlates positively with poor prognosis, tumor grade, invasiveness and drug-resistance. Metastatic canine mammary tumors display increased levels of p21 in the primary tumors but also in their metastases, despite increased cell proliferation [6]. We have previously shown that p21 can direct cells into the growth suppressive or anti-apoptotic pathways [3]. p21 has also been shown to be a prognostic marker indicating worse survival when cytosolically located in RCC [7]. Attenuation of p21 levels in RCC cell lines *in vitro* has been shown to sensitize the cells to apoptosis in response to conventional DNA damaging chemotherapy [8]. In addition, studies have demonstrated that forced cytosolic localization of p21 prevents apoptosis [5] and promotes growth in different cell types. Furthermore, p21 induction has been shown to be an early event in oncogenesis [9]. We have previously demonstrated an anti-apoptotic effect of p21 in RCC cell lines [8,10]. This survival function of p21 likely maintains cell viability during the DNA repair process [3], such that attenuation of p21 can subvert this repair process and thereby sensitize RCC to conventional chemotherapy [8].

“Inhibition of p21 for potential cancer therapy has the advantage of keeping p53 intact.”

Few small molecule inhibitors of p21 have been reported so far. These include butyrolactone I (BL) [11], LLW10 [12], sorafenib [13] and recently reported UC2288 [14]. BL is a competitive inhibitor of ATP for binding and activation of CDK. BL is a potent inhibitor ($IC_{50}=75\text{ }\mu\text{M}$) of endogenous p21 protein expression which is p53-independent [11]. It induces p21 degradation via the proteosomal pathway that precedes BL's inhibitory effects on the cell cycle. Moreover, when cancer cells exposed to DNA damaging agents, BL blocks the p53-dependent increase of p21 protein expression, reduces the G1 arrest and leads to a greater G2/M arrest as compared to the non-BL exposed cells.

We have previously discovered a small molecule p21 inhibitor, LLW10, using a one-bead one-compound combinatorial chemistry approach [12]. Rather than causing inhibition of p21 activity, LLW10 binds to p21 and induces ubiquitination and proteosomal degradation of p21 and consequent sensitization of chemotherapy-induced apoptosis in two RCC cell lines. However, LLW10 required high concentration ($\sim 100\text{ }\mu\text{M}$) to attenuate p21. In addition, LLW10 proved to be unstable, therefore it is not a good drug candidate to be developed for ultimate clinical use. We have recently demonstrated that sorafenib decreases levels of p21 independently of the mitogen-activated protein extracellular kinase (MEK)/extracellular signal-regulated kinase (ERK) and soluble epoxide hydrolase (sEH) pathways [13]. In cells treated with doxorubicin to augment p21, sorafenib markedly decreases p21, and the combinations of paclitaxel or doxorubicin with sorafenib show additive cytotoxicity, suggesting that sorafenib can act as a sensitizing agent for conventional chemotherapeutics in the treatment of RCC by attenuating p21.

More recently, have we identified another small molecule inhibitor of p21, UC2288, through modification of sorafenib [14]. But unlike sorafenib, UC2288 does not inhibit Raf kinases or alter p-ERK protein levels. UC2288 attenuates p21 protein at the level of transcription or post-transcription, and not via protein degradation. In addition, UC2288 markedly decreased

cytosolic p21 protein level ($IC_{50}=10\text{ }\mu\text{M}$) but not nuclear p21 levels, and inhibits RCC growth in association with attenuated cytoplasmic p21. It is important to note, different from BL, UC2288 functions independently of p53, a property that could be clinically very important as p53 is truly the “guardian of the genome” and thus its levels and function should be preserved.

“Small molecule inhibitors of cytoplasmic p21 could serve as novel drugs to treat chemo-resistant kidney cancer as well as other cancers.”

Cytoplasmic p21 conveys an anti-apoptotic function in many tumor cells through its induction by the DNA damage responsive p53 pathway, such that attenuation of p21 sensitizes cancer cells to DNA-damaging chemotherapy. Given the high levels of resistance of RCC to conventional chemotherapies, further development of small molecule inhibitors of p21, especially cytosolic p21, may lead to a novel approach for chemotherapy-resistant cancers including kidney cancer. Indeed, attenuation of cytosolic p21 has been suggested as a novel approach for RCC [15]. In this study, inhibition of CRM1, a nuclear exporter protein, confined p21 to the nucleus, resulting in downregulation of cytosolic p21 and upregulation of nuclear p21 in RCC, resulting in apoptosis induction and growth inhibition of RCC both *in vitro* and *in vivo*. While they both result in p21 attenuation, the efficacy of the CRM1 inhibitor KPT-330 is better than that of sorafenib, the latter of which attenuates both cytosolic and nuclear p21. However, the CRM1 inhibitor suggested in this study is not a specific inhibitor to cytosolic p21 but also has effect on other CRM1 target proteins such as p53.

“Future directions for p21 inhibitors.”

p53-independent small molecule inhibitors like UC2288 are especially attractive and worthy of further development to improve potency, specificity, and pharmacokinetic properties as well as to reduce toxicity. There are many conceivable applications of p21 inhibitors and promising possibilities for clinical translation as will be briefly discussed below.

Due to the nature of the differential effects of p21 as a function of its subcellular localization, it is likely that the most successful efforts to target p21 in cancer should have to keep this in mind. For example, targeting cytosolic p21 is much more likely to be a successful cancer chemotherapy approach than global inhibition of p21, at least in terms of specificity towards apoptosis. However, while such a strategy is not readily available at present, there are several studies showing that global p21 attenuation can decrease cell growth and promote apoptosis [16]. The most logical method employing p21 as chemotherapeutic adjunct at present would be to attach small molecule inhibitors to a tumor-targeting peptide, such as RGD; such integrin targeting tripeptides have shown some clinical utility in malignancies which display the selective integrin pair on their cell surfaces [17].

While antisense oligodeoxynucleotides and siRNA to p21 are commercially available for *in vitro* use, they have proven difficult to translate to the clinic due to insufficient efficacy, transient expression, and resistance [18]. This failure most likely relates to poor intracellular delivery of siRNA into target cells, and the rapid degradation of these oligomers by cellular nucleases. In addition, these approaches may be problematic because attenuation of p21 in all tissues, due to the location of p21 downstream of p53, may lead to an increased risk of

malignancy. On the other hand, transient decrease in p21, as could be done immediately prior to a chemotherapeutic burst, could be successful and in this case the transitory nature of antisense p21 could be beneficial. Other approaches include targeting antisense or small molecule inhibitors to cancers using antibody-associated liposomes; this approach has been used with other compounds and antibodies with modest success [19]. The advantage of this approach would be specificity towards the cancer of interest, but the disadvantage would be non-specificity of intracellular downregulation of p21.

It is likely that the best clinical utility of p21 inhibition would lie in the use of these compounds temporally around the time of infusion of either DNA-damaging chemotherapy or the administration of radiation, because a primary role of p21 in cancer is to prevent apoptosis. Thus, inhibitors of p21 may be used as sensitizers to increase the ability of conventional treatments currently used in clinic, especially when the presence of p21 interferes with cell death.

In summary, the use of specific inhibitors of the cyclin dependent kinase inhibitor p21 has tremendous potential as a new paradigm for the treatment of a variety of cancers as well as for use as a tool in exploring mechanisms of cell cycle arrest and apoptosis. Small molecule inhibitors of p21 have distinct advantages including cell permeability, increased stability, ease of manufacture and oral delivery.

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