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Insights Into the Broad Cellular Effects of Nelfinavir and the HIV Protease Inhibitors Supporting Their Role in Cancer Treatment and Prevention

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Abstract

Purpose of Review—The development of HIV protease inhibitors (PIs) more than two decades ago heralded a new era in HIV care, changing the infection from universally-fatal to chronic but controllable. With the widespread use of PIs, there was a reduction in the incidence and mortality of HIV-associated malignancies. Studies later found these drugs to have promising direct antitumor effects.

Recent findings—PIs have a wide range of effects on several cellular pathways that are important for tumorigenesis and independent of inhibition of the HIV protease, including reducing angiogenesis and cell invasion, inhibition of the Akt pathway, induction of autophagy, and promotion of apoptosis. Among PIs, Nelfinavir appears to have the most potent and broad antineoplastic activities, and also affects replication of the oncogenic herpesviruses Kaposi sarcoma-associated herpesvirus and Epstein-Barr virus. Nelfinavir is being studied for the prevention and treatment of a wide range of malignancies in persons with and without HIV infection.

Summary—Nelfinavir and other PIs are safe, oral drugs that have promising antitumor properties, and may prove to play an important role in the prevention and treatment of several

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cancers. Additional insights into PIs' mechanisms of action may lead to the development of novel cancer chemotherapy agents.

Keywords

HIV protease inhibitor; nelfinavir; cancer; Kaposi sarcoma; treatment

Introduction

Protease inhibitors (PIs) were specifically designed to inhibit the HIV aspartyl protease, but serendipitously were found to have effects that confer broad antineoplastic and antiviral activity. This led to the idea that PIs could be efficiently repositioned for cancer chemotherapy [1,2], especially in light of their oral bioavailability, wide clinical use, and minimal toxicity. In particular, nelfinavir, now rarely used for antiretroviral therapy (ART), has emerged as the most potent candidate PI for many cancers in patients with or without HIV infection. This review provides an update on the use of PIs for cancer, with an emphasis on nelfinavir, including information on the antitumor mechanisms that have been identified, completed and ongoing clinical trials, and priorities for future research.

Description of HIV protease inhibitors

Protease inhibitors were developed to block the maturation of the HIV virion, by inhibiting the viral aspartyl protease's cleavage of precursor polyproteins into their functional forms. Using the HIV protease crystal structure, specific small molecule inhibitors were rationally designed, leading to FDA approval of saquinavir for ART in 1995. Since that time, 9 additional HIV PIs have been approved: ritonavir, indinavir, nelfinavir, amprenavir, lopinavir, atazanavir, fosamprenavir, tipranavir, and darunavir. The use of PIs, in combination with available reverse transcriptase inhibitors, rapidly became the standard of care, and ushered in the era of "highly active" ART. Immediately afterward, mortality due to HIV infection began to decline, the incidence of opportunistic infections plummeted, and the number of new cases of AIDS-defining cancers (especially Kaposi sarcoma, KS) was markedly reduced.

Nelfinavir

The PI nelfinavir mesylate was approved in 1997, initially at a dose of 750 mg thrice daily, and later at 1250 mg BID. Serious adverse effects of nelfinavir are uncommon, though up to 30% of patients experience dose-dependent diarrhea [3]. Nelfinavir has subsequently been shown to be less effective against HIV than ritonavir-boosted PIs, and is no longer widely used for ART [4].

Evidence for anti-cancer activity of PIs in HIV-infected patients

Co-incident with the widespread use of PIs came a series of case reports demonstrating the regression of KS with PI-based ART alone [5-13]. However, as potent new non-nucleoside reverse transcriptase inhibitors (NNRTIs) were introduced, case series suggested that regression of KS was dependent more on the potency of the regimens to suppress HIV replication and reconstitute T cell immunity than on the individual drugs used [14-16].

Similarly, no difference has been seen in the reduction of risk for incident KS among HIV patients between those receiving regimens containing NNRTIs vs. PIs [17,18], including one study with a small number of KS cases which specifically looked at nelfinavir [19]. Though it is clear that all effective ART substantially reduces the risk of AIDS-defining cancers, to date no adequately powered randomized trial has evaluated the relative benefits of nelfinavir or other PIs for the treatment or prevention of KS or other malignancies in HIV-infected individuals.

In vitro and preclinical in vivo studies relevant for oncology

PIs have a broad range of effects on human cells that cause toxicity [4], but also confer potent anticancer properties (Table 1). Despite their limited homology with the HIV protease, cellular proteases appear to be the primary targets of PIs that account for their antitumor activity, particularly the proteasome and matrix metalloproteases (MMPs). However, numerous additional activities of PIs have been appreciated. A landmark paper by Gills et al [22] found that multiple PIs had activity against all 60 cell lines in the NCI60 panel, with nelfinavir being most potent among the drugs tested, and described multiple potential mechanisms of action against cancer, including endoplasmic reticulum (ER) stress, autophagy, apoptosis, and inhibition of Akt signaling. A comprehensive computational analysis of structural protein interactions found 92 predicted cellular targets of nelfinavir, of which the 7 with the strongest binding affinities were aspartyl proteases [59]. The list of remaining targets was dominated by protein kinases such growth factor receptors that are upstream regulators of Akt, NF κ B, and other signaling molecules that are known to be down-regulated by nelfinavir [40]. PIs also have effects that may be disadvantageous for cancer treatment, including increased expression of multidrug transporter ABCB1 (P-glycoprotein) in KS cells, which confers *in vitro* resistance to doxorubicin and paclitaxel [60]. Considerable variation in the cellular effects of individual PIs has been observed, which may be both concentration- and cell type-dependent, making it challenging to predict tumor-specific activities.

Angiogenesis and cell invasion

Initial anticancer studies of PIs found that indinavir and saquinavir were potent inhibitors of angiogenesis and tumor cell invasion in murine KS models by blocking activation of MMP2 [57], and subsequently that ritonavir and saquinavir inhibit MMP2 and MMP9 and block invasion of cervical intraepithelial neoplasia cells in an *in vitro* system [55]. Amprenavir was also shown to inhibit MMP2 in hepatocarcinoma cells and impair cell invasion and tumor xenograft growth in nude mice [56]. Additionally, PIs can block angiogenesis through down-regulation of signaling pathways, such as phosphatidylinositol 3-kinase (PI3K)/Akt, which modulates the expression of vascular endothelial growth factor (VEGF) and numerous other factors involved in neovascularization [27,44,56,61].

Inhibition of Akt

PI3K/Akt signaling and downstream mediators, such as mammalian target of rapamycin (mTOR) and VEGF, contribute to oncogenesis through effects on multiple cellular processes, including proliferation, motility, angiogenesis, transformation, apoptosis/survival,

and DNA repair (reviewed in [62,63]). Upregulation of PI3K/Akt signaling protects against apoptotic cell death, and thereby confers resistance to radiation and chemotherapy in a number of cancers [63]. PIs inhibit phosphorylation of Akt in multiple tumor cell lines [20-22,25]. Nelfinavir appears to be the most potent inhibitor of Akt among the PI class [22], although this varies by cell type [25]. In rapamycin-resistant diffuse large B cell lymphoma lines in which Akt activation was upregulated, the combination of rapamycin with nelfinavir or the Akt inhibitor MK-2206 resulted in synergistic cytotoxicity [30]. A promising observational study of HIV-infected patients showed that those taking nelfinavir-based ART showed lower levels of Akt phosphorylation in leukocytes compared with patients on ART without a PI or not receiving antivirals; furthermore, nelfinavir was not associated with increased radiation toxicity [64]. However, the level of Akt inhibition does not always correlate with antitumor activity, and in some model systems nelfinavir paradoxically activates Akt [29,48]. The precise mechanism by which PIs prevent Akt phosphorylation by PI3K is unknown, but may result from inhibition of upstream growth factors, induction of ER stress, or other effects [29,31].

Endoplasmic reticulum stress

When accumulation misfolded proteins or other stresses overwhelm the ER equilibrium, the unfolded protein response (UPR) is triggered, which results in the attenuation of protein translation and cell cycle arrest (reviewed in [65,66]). Nelfinavir and other PIs cause ER stress [22,29,31,35-39]. In liposarcoma and castration-resistant prostate cancer cell lines (which has a lipogenic phenotype), nelfinavir induces overwhelming ER stress by inhibition of site-2 protease, resulting in impaired processing and accumulation of sterol regulatory element binding protein-1 (SREBP-1) and activating transcription factor 6 [38,43,67]. This fits well with what is known about a known toxicity of PIs, lipodystrophy, which appears to result from increased levels of SREBP-1 [1].

Proper protein folding is facilitated by chaperones, and degradation of misfolded proteins is performed primarily by the proteasome. As such, interference with either of these functions by PIs may contribute to ER stress [24,29,35,39,53]. Inhibition of 20S proteasome activity by ritonavir [44,54,58] and saquinavir [50] has been reported. Nelfinavir also has cell type-dependent activity against the proteasome [24,25,29,48,54]. In one study, however, nelfinavir partially inhibited proteasome activity in breast cancer cell lysates, but caused ER stress differently from proteasome inhibitors [29]. Rather, the authors report evidence that the principal target of nelfinavir in these cells appeared to be a chaperone, heat shock protein 90, leading to ER stress as well as disruption of Her2 and Akt signaling. ER stress and cell killing can be increased by the combination of a PI (nelfinavir or ritonavir) and the proteasome inhibitor bortezomib in multiple cancer types (including those resistant to bortezomib alone) based on *in vitro* and mouse models [24,25,32,39,68,69]. ER stress and the UPR lead to autophagy, which results in cell survival if re-equilibration can be established, or in apoptosis and cell death in cases of overwhelming ER stress.

Autophagy

Autophagy is a catabolic process in which proteins and organelles are degraded and recycled either as a normal part of homeostasis, or in order to survive a period of nutrient starvation

(reviewed in [70]). In addition to nutrient starvation, autophagy can be triggered by ER stress/UPR or PI3K/Akt inhibition caused by PIs or cancer chemotherapeutic agents [40]. Conversely, interfering with autophagy can cause ER stress and lead to cell death. As such, autophagy may protect cancer cells against death, and strategies that antagonize autophagy may complement the activity of therapies that induce ER stress/UPR. For example, an inhibitor of autophagy (3-methyladenine) increased nelfinavir-induced cell death in a panel of cancer cell lines [22]. In triple-negative breast cancer cell lines, the cytotoxic effects of ER stress induced by nelfinavir and/or celecoxib were synergistically enhanced by chloroquine, an inhibitor of autophagy [41]. Nelfinavir caused ER stress and autophagy in primary chronic lymphocytic leukemia cells from 14 patients; however, cytotoxicity was not observed unless nelfinavir was combined with chloroquine [42]. Apoptosis of prostate cancer cells treated with nelfinavir was also additively increased by hydroxychloroquine [43].

Apoptosis

PIs induce cell death through apoptosis in a variety of different cell lines [21,33,34,36,44-46,53,54], mediated either through ER stress or by other mechanisms, including inhibition of signaling through Akt, STAT3, c-Src, and NF κ B [21,49]. PIs may inhibit NF κ B activation through inhibition of proteasomal degradation of I κ B [44,46,49,51,52]. Again, effects appear to be cell type-dependent; for example, interestingly, ritonavir may be protective against apoptosis in normal primary T cells [71].

Effects of nelfinavir on oncogenic herpesviruses

Nelfinavir, but not other PIs, is a potent inhibitor of Kaposi sarcoma-associated herpesvirus (KSHV; human herpesvirus 8) replication in vitro with an EC₅₀ 10 μ M [72]. Nelfinavir does not act on the herpes protease (unpublished data); rather, its cellular effects appear to interfere with production of infectious KSHV. Given the importance of KSHV replication for KS pathogenesis, it is possible that nelfinavir could confer benefit through its antiviral as well as its antitumor properties [73]. Interestingly, nelfinavir also appears to trigger reactivation of KSHV and EBV in latently infected lymphoma cell lines, through induction of ER stress [69,74]. Nelfinavir's effects on viral reactivation were independent of cytotoxicity. However, viral reactivation induced by nelfinavir in tumors might result in increased immune destruction and/or activity of nucleoside analog antivirals. Alternatively, increased expression of early viral genes like KSHV vIL-6 could have adverse consequences [75].

Clinical oncology trials (Table 2)

Based on their pre-clinical findings, Monini et al conducted a pilot trial of indinavir for endemic (HIV-negative) Kaposi sarcoma [76]. Twenty-eight patients received 800 mg of indinavir BID for 12 months, and although a control group was not included, clinical response was associated with blood levels of indinavir.

Two phase I studies of nelfinavir combined with chemoradiotherapy have been performed (Table 2). In patients with pancreatic cancer, nelfinavir 1250 mg BID was added to

gemcitabine, cisplatin, and radiation [77] for its Akt inhibitory and radiosensitization properties. The authors reported that none of the observed toxicities appeared attributable to nelfinavir. Dose escalation was not pursued, because Akt phosphorylation in leukocytes was already reliably achieved at the standard nelfinavir dose. In patients with unresectable non-small-cell lung cancer, nelfinavir was studied at 625 mg BID or 1250 mg BID, in combination with cisplatin, etoposide, and radiation [78]. Again, no dose-limiting toxicities were observed and no toxicities were attributed to nelfinavir. The authors concluded that 1250 mg BID was an appropriate dose for further study with concurrent radiation chemotherapy.

In two phase I dose-escalation trials, nelfinavir has been studied as a single agent (**Table 2**). Among 11 evaluable patients with refractory solid tumors, nelfinavir was increased from 1250 mg BID to 3125 mg BID until the maximum tolerated dose was reached or there was disease progression [79]. No grade 4 or 5 toxicities were observed, and assays of peripheral blood mononuclear cells generally showed inhibition of Akt activation and increased markers of ER stress and apoptosis at every dose level. In patients with recurrent liposarcomas not amenable to surgical therapies, nelfinavir was administered orally BID for a 28 days/cycle in a phase 1/2 design [80]. Grade 3 pancreatitis requiring hospitalization was encountered at the lowest dose level, and reversed within 3 days; none of the pre-specified dose-limited toxicities were observed. Of note, pharmacokinetic data suggest that there may be only minimal increases in plasma drug concentrations with nelfinavir doses above 1875 mg BID, and that the drug induces its own clearance at doses of 4500 mg BID.

In sum, these trials show that nelfinavir: i) inhibits Akt-phosphorylation in blood leukocytes at standard doses in patients with and without cancer, ii) appears to be well tolerated even when administered with cytotoxic chemotherapy agents and concomitant radiation therapy, iii) may be administered as a single agent at higher than standard doses with acceptable toxicity, and iv) plasma levels may plateau with increasing doses and even decrease at very high doses. These observations and the preclinical data described above have motivated several ongoing clinical trials, including several phase 1/2 studies of patients with brain tumors, cervical cancer, pancreatic cancer, myeloma, lung cancer, and KS.

Conclusions

Epidemiologic and clinical studies of HIV-associated malignancies has led to recognition of the potential role for PIs in the treatment and prevention of a wide range of cancers. While as a class PIs may act similarly against HIV, they appear to be more heterogeneous in their effects on specific cellular pathways related to oncogenesis and inhibition of non-HIV viral production. Nelfinavir appears to be the most promising PI for the prevention and treatment of cancer, and a number of small clinical trials have begun to be conducted, showing encouraging tolerability and activity of nelfinavir-containing chemotherapeutic regimens for several cancer types in HIV-negative patients. Nelfinavir is highly attractive as a new cancer drug given that it is orally bioavailable and has an excellent safety profile, with more than 15 years of experience in of wide use in patients with HIV infection (including adults, children, and pregnant women, in resource-rich and resource-limited regions). These characteristics raise the possibility of novel chemopreventative strategies using nelfinavir in selected high

risk settings, such as prevention of viral-associated malignancies, prevention of various cancers in chronically immunosuppressed patients, and secondary prevention of relapses.

Future studies should continue to define the differential antitumor effects among the different PIs, the basis for nelfinavir's apparently superior activity, and the degree to which this varies by cell type. Such information would further the selection of the most synergistic combinations of chemotherapeutic drugs and the most appropriate tumors to target. In addition, an increased understanding of the PIs' mechanisms of actions could lead to the development of PI analogs or derivative agents with greater potency, and result in more effective treatments for a wide range of cancers and viral infections.

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Key Points

- PIs have been shown to kill a wide range of cancer cells types and inhibit angiogenesis in a large number of preclinical studies.
- The mechanisms of action for these antitumor properties appear numerous, and include inhibition of Akt signaling, MMPs, and the proteasome, and induction of ER stress, autophagy, and apoptosis.
- Nelfinavir appears to be the have the most broad and potent antitumor properties among the PIs, and has also been shown to have unique effects on oncogenic herpesvirus replication *in vitro*.
- Nelfinavir is orally bioavailable, and has been shown through extensive use to be safe, and could be rapidly and efficiently repositioned as a cancer chemotherapeutic drug.
- Early clinical studies are promising, but controlled trials are needed to evaluate the efficacy of nelfinavir against cancers in HIV-infected as well as uninfected patients.

Table 1
Antitumor mechanisms of HIV protease inhibitors

Host cell effect	Protease inhibitor(s)	Cell types	References
Inhibition of Akt	nelfinavir, ritonavir, lopinavir, saquinavir, amprenavir	multiple myeloma, pituitary adenoma, non-small cell lung cancer, head and neck squamous cell carcinoma, urinary bladder carcinoma, prostate cancer, glioma, breast cancer, diffuse large B cell lymphoma	[20-31]
ER stress	nelfinavir, ritonavir, atazanavir	cervical cancer, renal cancer, glioma, breast cancer, non small cell lung cancer, head and neck squamous cell carcinoma, ovarian cancer, liposarcoma, multiple myeloma	[22,29,31-39]
Autophagy	nelfinavir	non small cell lung cancer, breast cancer, prostate cancer, chronic lymphocytic leukemia	[22,40-43]
Apoptosis	nelfinavir, saquinavir, ritonavir, lopinavir	KS cell lines, breast cancer, non small cell lung cancer, hepatocellular carcinoma, cervical cancer renal carcinoma, Adult T cell leukemia, T cell lymphoma, EBV-positive B cell lymphoblastoid, prostate, glioblastoma multiforme, Jurkat leukemia, multiple myeloma, ovarian cancer, neuroblastoma, melanoma, Burkitt lymphoma, fibrosarcoma, myeloid leukemia	[21,22,24,26,32,34,36,44-54]
Matrix metalloprotease inhibition	indinavir, ritonavir, saquinavir, amprenavir	Cervical carcinoma/CIN; HCC	[55-57]
Inhibition of NFkB	ritonavir, saquinavir	KS cell lines, Adult T cell leukemia, EBV-positive B cell lymphoblastoid, neuroblastoma	[44,46,49,52]
Proteasome inhibition	saquinavir, nelfinavir, lopinavir, ritonavir	Prostate, multiple myeloma, melanoma, cervical cancer, breast cancer, head and neck squamous cell carcinoma, murine T lymphocytes, T lymphoma	[24,25,29,31,47,48,50,54,58]

Table 2
Clinical oncology trials of HIV protease inhibitors

Trial	PI and maximum dose	PI-related toxicities	Reference
Endemic (HIV-negative Kaposi sarcoma)	Indinavir, 800 mg BID	Occasional modest skin and renal adverse effects reported	[76]
Pancreatic cancer (concomitant chemoradiotherapy with cisplatin, gemcitabine and radiation)	Nelfinavir, 1250 mg BID	Many toxicities associated with concomitant chemoradiation therapy, none attributed specifically to nelfinavir	[77]
Lung cancer (concomitant chemoradiotherapy with cis platin, etoposide and radiation)	Nelfinavir, 1250 mg BID	Many toxicities associated with concomitant chemoradiation therapy, none attributed specifically to nelfinavir	[78]
Refractory solid tumors	Nelfinavir, 3125 mg BID, dose escalation ongoing	ALT, AST transaminitis, diarrhea, and hyperglycemia. None considered dose limiting.	[79]
Liposarcoma	Nelfinavir, 4250 mg BID	1 grade 3 pancreatitis (reversed within 3 days), no other grade 3 or 4 toxicities; diarrhea, elevated AST, ALT; elevated glucose, elevated triglycerides	[80]