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Commentary

Nelfinavir

A magic bullet to annihilate cancer cells?

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Chemotherapy resistance remains a major obstacle for successful cancer treatment. Thus, the continuous development of new anticancer drugs could help address drug resistance by offering a broader spectrum of alternative anticancer agents. The discovery of new anticancer agents increasingly relies on high-throughput screening in conjunction with systems biology.[1] Yet, the process of evaluating ADME (absorption, distribution, metabolism and excretion) and toxicity remain lengthy processes not shortened by the new massively-parallel technologies. However, the strategy of repositioning FDA approved drugs for cancer treatment as a new indication offers a rapid and inexpensive approach to developing alternative anticancer agents, benefiting from the known pharmacokinetic and toxicological properties.[2] Currently numerous novel, target-selective drugs originally not developed for cancer therapy are being studied for a potential role in cancer treatment including, cyclooxygenase-2 inhibitors, the oral hypoglycemic rosiglitazone, the immunosuppressant rapamycin, and many others.[3-6]

One promising class of drugs with potential anticancer activity belongs to the family of protease inhibitors that were originally utilized for antiviral therapy against HIV. For instance, Nelfinavir (Viracept®) has drawn attention due to its potent activity in tumor cell suppression. Nelfinavir contains a unique cis-decahydroisoquinoline-2-carboxamide moiety, which may provide the structural basis for its increased efficacy against cancer compared to other HIV protease inhibitors.[7] Early evidence indicates that Nelfinavir and other HIV protease inhibitors enhance the sensitivity of tumor cells to radiotherapy in vitro and in vivo.[8] It has also been reported that Nelfinavir (at the therapeutic concentration for HIV treatment) suppresses growth of several cancer cell lines and xenografts.[7] In this issue of *Cancer Biology & Therapy*, Brüning et al. report that

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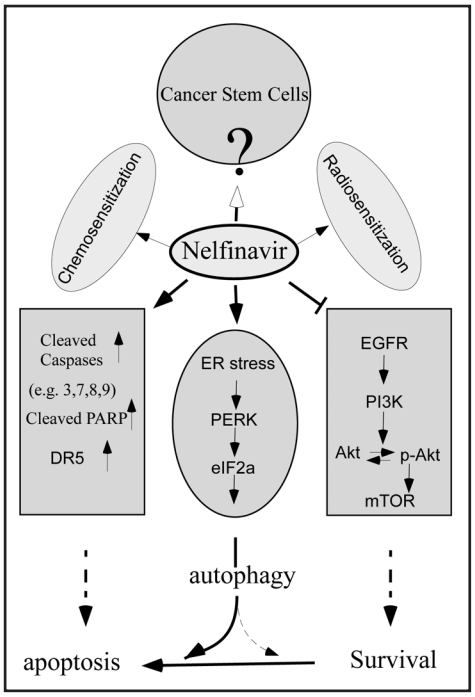

Figure 1. The diagram depicts the possible mechanisms of action of Nelfinavir against cancer cells. Nelfinavir triggers typical cell death by activating caspases (e.g., caspase-3, 4, 8, 9) as well as inducing sustained ER stress and autophagy. In addition, Nelfinavir inhibits the EGFR and its downstream component activity (e.g., PI-3K/Akt). The sum effect is to reprogram the cancer cell for self-destruction. ER: Endoplasmic Reticulum. EGFR: Epidermal Growth Factor Receptor.

Nelfinavir inhibits cultured and ex-vivo ovarian cancer cell proliferation and induces cell death regardless of the carboplatin-resistance status.[9] The average concentration of Nelfinavir exhibiting 50% inhibition of cultured cell growth and ex-vivo ovarian cancer cells was ~5 μmol/L and ~10 μmol/L, respectively. Such concentrations correspond to the C_{max} (~7 to 9 μmol/L) for Nelfinavir in HIV patients.[10] It has been suggested that the anticancer effects of Nelfinavir may be achievable for cancer patients as well.[11] This is important because ovarian cancer is primarily treated by a combination of carboplatin and Taxol. Unfortunately, ovarian cancer cells, like most cancers, acquire chemo-resistance rapidly, resulting in recurrence and a high mortality.

The mechanism underlying anti-cancer drug resistance is not fully understood. One possibility, the "cancer stem cell" hypothesis, is that the cancer initiating cells that exhibit stem cell properties also share the inherent tolerance of stem cells to toxins.[12] Given the effects of Nelfinavir on carboplatin resistant ovarian cancer cells, it is reasonable to postulate that Nelfinavir may target cancer stem cells, which opens an interesting direction for further investigations into Nelfinavir's anti-tumor effects in the new context of "cancer stem cells". Of note, a high dose of Nelfinavir alone was shown to yield results that are comparable with the anticancer effect of Taxol on OVGH1 and OVGH5 ovarian cancer cells, although a negative effect was observed when Nelfinavir was combined with Taxol. However, Nelfinavir alone had no measurable effects on the viability of fibroblasts or peripheral blood mononuclear leukocytes.[13] Thus, Nelfinavir may provide an alternative modality, even as a single-agent chemotherapy, for ovarian cancer.

Nelfinavir may exert its anti-cancer effect via several mechanisms of action as summarized in Figure 1. Similar to most other anticancer drugs, Nelfinavir triggers caspase-dependent cell death. Bruning et al. in another study using antibody array analysis demonstrated that Nelfinavir induces TRAIL receptor (DR5) expression and can thus sensitize ovarian cancer cells to TRAIL treatment.[13] Nelfinavir possesses a unique mechanism that permits the induction of caspase-independent cell death, endoplasmic reticular (ER) stress and autophagy.[7,14] Consistent with previous observations, Bruning et al. demonstrated that Nelfinavir induces vacuoles in the carboplatin-resistant ovarian cancer OVCAR3 cells and that the aggregated vacuoles specifically co-localized with the ER membranes, but not with lysosomes or liposomes. Furthermore, ER stress markers, such as BiP and phosphor-eIF-2 expression, increased following treatment with Nelfinavir. Therefore, targeting the ER stress pathway and thereby shifting the balance between survival and apoptosis using a sustained ER stress inducer (e.g., Nelfinavir) could become an attractive pharmacological strategy for suppressing cancer cells.

Another mechanism through which Nelfinavir may act against cancer is via inhibition of PI-3K/Akt activation by targeting EGFR or IGFR.[7] EGFR/ERBB2/PI-3/Akt is a common survival-signaling pathway in most cancer cells including ovarian cancer. To better understand how Nelfinavir inhibits EGFR signaling, computer simulation of drug-target interaction can be used. Such analysis can provide valuable information for the future development of similarly acting compounds. As Figure 2 shows, the Nelfinavir molecule (red) is able to dock to the EGFR/ERBB1 protein in its ATP-binding domain. A comparison is made between the docked Nelfinavir molecule and a known dual inhibitor against both EGFR/ERBB1 and ERBB2, Lapatinib (gold), which has been co-crystallized with the EGFR/ERBB1.[15] The Lapatinib molecule extends deep into the active site where it is engaged in strong interactions with the protein while the Nelfinavir molecule barely fits into the site due to its larger number of rings, resulting in more steric hindrance as it slides into the cavity. The best conformer of Nelfinavir with EGFR found by a virtual docking study as compared to Lapatinib is shown in Figure 2. However, the precise interaction between Nelfinavir and EGFR or ERBB2/Her2/Neu (or any mutated forms) remains to be solved by crystallization studies.

In summary, the preclinical research conducted by Brüning et al. is another example supporting the burgeoning strategy of screening drugs, which are FDA-approved for non-oncologic applications, for oncologic purposes as a first step towards evaluating their off-label use in cancer therapy. Nelfinavir is currently being tested in cancer patients in a phase I clinical trial that aims to evaluate the use of Nelfinavir as a single agent or in combinational treatment.[11,16] The short-cut in drug development afforded by retesting in vitro the anticancer potential of drugs already FDA approved may accelerate the stream of new compounds for use in oncology, hence broadening the spectrum of alternative choices in chemotherapy. At the moment, this brute-force strategy may be our best bet in the race against the unfathomable potential of cancer cells to develop drug resistance.

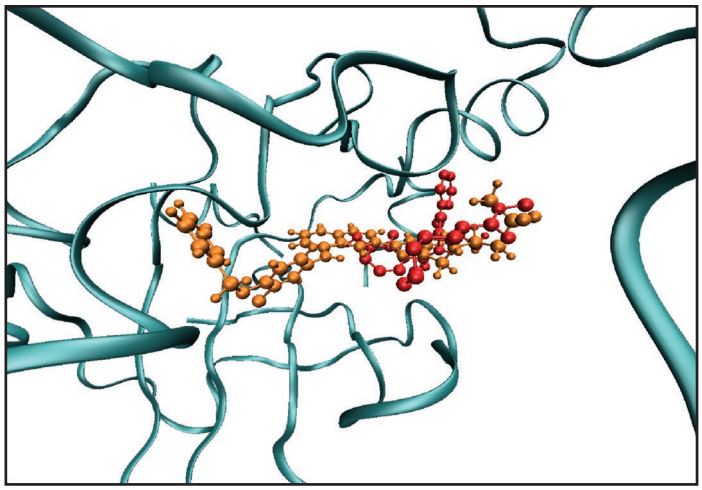

Figure 2. The EGFR (ERBB1) protein is drawn in ribbons while the Nelfinavir and Lapatinib molecules are portrayed in the ball-and-stick model and colored in red and gold, respectively. The geometry of the Nelfinavir molecule was first optimized using the deMon2k software (http://www.demon-software.com) before the virtual docking investigation. Then the optimized structure of Nelfinavir served as the starting point in its automated docking with the target protein ERBB1 (PDB entry: 1XKK) with Autodock 4.0.[17] The ATP binding domain was defined as the docking site where Nelfinavir interacted with the protein. The predicted binding free energy was employed as the criterion for the best conformer.

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