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Inhibition of tumor cell invasion and ErbB2/PI3K signaling pathways by the glutathione disulfide-mimetic NOV-002

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Abstract

NOV-002, a glutathione disulfide-mimetic, is in advanced clinical development for oncology indications. It is believed to act by modulating intracellular and cell surface redox status resulting in a pleiotropic pharmacologic profile. Here we report the ability of NOV-002 to inhibit the invasiveness of human tumor cells *in vitro* by suppressing the activation of a well-characterized, redox sensitive signaling pathway that is critical for tumor cell growth and invasion. In the Matrigel invasion assay, NOV-002 inhibited the invasion of a variety of human tumor cell lines including A549 (non-small cell lung), MDA-MB436 (breast), SKOV3 (ovarian) and HCT-15, HCT-116 and Colo205 (colorectal) in a dose-dependent manner with IC50s between 17.7μM and 91μM. In contrast, cell migration was only inhibited in the colorectal tumor cell lines suggesting that NOV-002 may affect pathways which are specific for invasion. 1 mM NOV-002 (the highest concentration tested in the invasion and migration assays) was not toxic to any of the tumor cell lines studied even after 72 hours of culture using MTT viability assay. These effects were shown to involve the suppression of ErbB2 and phosphoinositide-3 kinase (PI3K) pathways by NOV-002. Immunoblot analysis demonstrated that NOV-002 reduced the expression of the phosphorylated (active) forms of signaling proteins ErbB2 and PI3k, known to regulate tumor cell invasion in A549 and Colo205 cells, without affecting the total amount of these proteins. Activation of Akt and RhoA, downstream molecules of the ErbB2/PI3K pathway, was also inhibited by NOV-002. Thus in both A549 and Colo205 cell lines, the active form of both upstream (ErbB2 and PI3K) and downstream (Akt and RhoA) signaling proteins were suppressed by NOV-002 at concentrations between 30 mM and 1 mM in a dose-dependent manner. Inhibition was more pronounced at 24 hours post-exposure compared to 8 or 16 hours. In prostate cancer PC3 cells which are not affected by NOV-002 in the invasion assay, suppression of ErbB2/PI3K signaling pathway was not observed. Thus, inhibition of tumor cell invasion by NOV-002 may be due to the inhibition of the ErbB2/PI3K signaling pathway activation that controls this process. We have previous shown that ERp5, a member of the redox-regulated protein disulfide isomerase family, promotes tumor cell invasion by activating the ErbB2/PI3K pathway. Thus ERp5 may represent a direct target for NOV-002. These results also indicate that NOV-002 may have particular therapeutic benefit in the treatment of cancers that utilize ErbB2 and PI3K pathway activation.

NOV-002 Background

- The active ingredient in NOV-002 is **oxidized glutathione**
- Changes in the ratio of oxidized: reduced glutathione controls cellular redox state and can regulate protein function by the reversible formation of mixed disulfides between protein cysteines and glutathione (= **glutathionylation**).
- Protein glutathionylation by NOV-002 results in pleiotropic effects on cell functions including cell signaling pathways, cytoskeletal architecture and cytokine production and is associated with hematopoiesis, immune stimulation and increased chemosensitivity of tumor cells.
- NOV-002, in combination with standard chemotherapy, is also the subject of an ongoing pivotal Phase 3 trial in advanced non-small cell lung cancer and two phase 2 trials: (i) in combination with doxorubicin-cyclophosphamide and docetaxel as part of neoadjuvant treatment of breast cancer; and (ii) in combination with carboplatin in platinum refractory ovarian cancer.

References

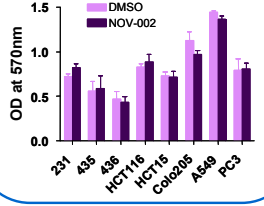
Townsend, DM et al. *Cancer Res* 2008; 68 (6): 2870-2877.

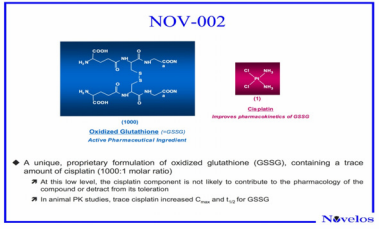
Townsend, DM et al. *Exp Opin Invest Drugs 2008; 17 (7): 1075-1083*

Krasner, CN et al. **J Clin Oncol 26: 2008 (May 20 suppl; abstr 5593)**

Figure 1 NOV-002 is Not Toxic to Cancer Cell Lines

MTT assay was performed 72 hours after the addition of 1mM (final concentration) of NOV-002 in cancer cells. No toxicity of NOV-002 on these cells was observed.





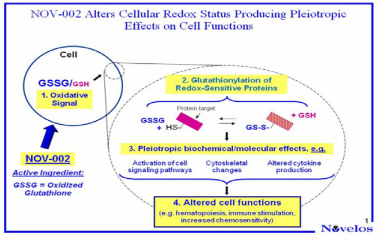


Table 1

NOV 002 suppresses the ErbB2/PI3K pathway

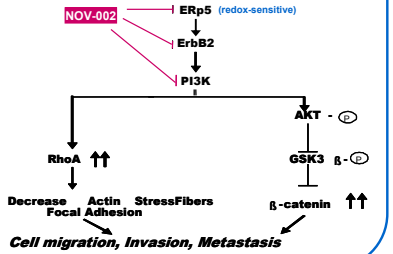


Figure 2 NOV-002 Suppresses Tumor Cell Migration

NOV-002 suppressed the migration of HCT15 and Colo205 in a dose-dependent manner. Migration assay was performed on these cell lines following the addition of NOV-002.

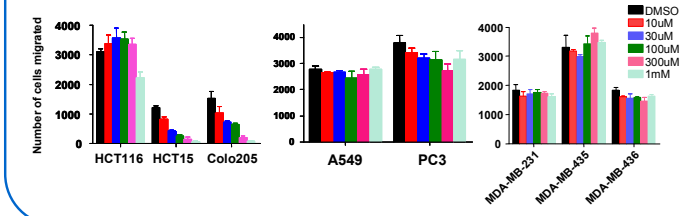


Figure 3 NOV-002 Suppresses Tumor Cell Invasiveness

NOV-002 suppressed the invasion of HCT116, HCT15, Colo205, MDA-MB-436 and A549 in a dose-dependent manner. Invasion assay was performed on these cell lines following the addition of NOV-002.

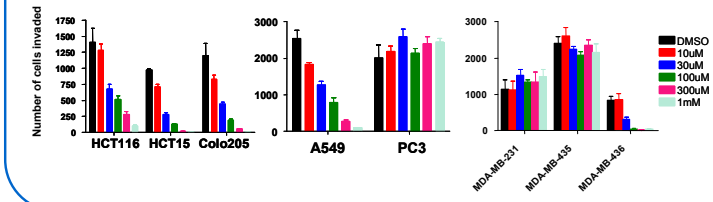


Figure 4 NOV-002 Suppresses ErbB2/PI3K Pathway Activity

NOV-002 reduces the expression of phosphorylated ErbB2 and PI3K whereas has no effect on the total protein expression of these two molecules.

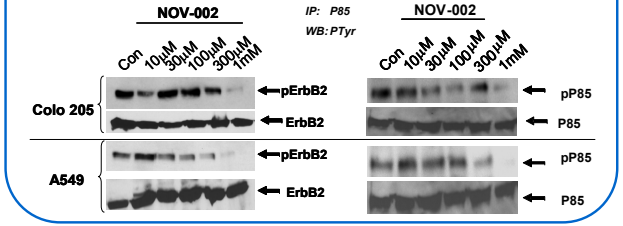
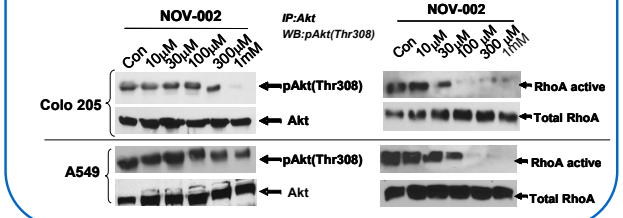


Figure 4 NOV-002 Reduces Akt and RhoA Expression

NOV-002 reduces the expression of active form of Akt and RhoA whereas has no effect on the total protein expression of these two molecules.



Conclusions

- **NOV-002 is not toxic to cancer cell lines.**
- **NOV-002 specifically interferes with invasion process of cancer cell lines.**
- **NOV-002 suppresses the ErbB2/PI3K pathway.**
- **NOV-002 reduces the expression of active Akt and RhoA.**