Selinexor Down Regulates Expression of DDR Proteins in Solid and Hematological Cancer Cells Where More Reduction Observed in More Sensitive Cell Lines

Abstract

Background: SINE™ is a family of small-molecule drugs that inhibit Exportin 1 (XPO1/CRM1) nuclear export, resulting in the accumulation of tumor suppressor protein (TSAP) such as p53, FODD, p53D and p57 in the nucleus and subsequently leading to specific cancer cell death. Selinexor (KOP-6002) is a clinical SINE™ compound currently in human phase I clinical trials in patients with solid and hematological malignancies. This study was conducted to evaluate the effects of selinexor on DNA repair mechanisms and to test the cytotoxic effects of combining selinexor with DNA damaging agents on hematological and solid tumors.

Methods: Whole protein extracts of cell lines from solid and hematological cancer cell lines treated with selinexor with or without agents that induce DNA damage were analyzed by Western Blot Analysis (WB), immunofluorescence and quantitative PCR. Selinexor treated cells from solid and hematological cancer cell lines were analyzed by immunofluorescence to evaluate DNA damage. Not-all-cells long cancer AS94 Xenografts were treated with selinexor (5 mg/kg or10mg/kg) and radiation (1 Gy) alone or in combination and tumor growth was evaluated for 22 days.

Results: Treatment of solid and hematological cancer cell lines with selinexor did not induce DNA damage but increased the expression of DNA damage repair (DDR) proteins: MSH2, MLH1, PMS2, Rad51, and CtBP1. Selinexor regulates the expression of Chkl, Rad51, MSH6, MSH3 and MLH1 on the transcriptional level and PMS2 expression on the posttranslational level. There was a trend between the degree of DNA damage-repair-proteins reduction in sensitivity. Knock down of Chkl alone, induced cytoxicity whereas silencing of the other DDR proteins did not affect cell viability. Selinexor treatment following exposure to the DNA damaging agents decreased and silenced the repair mechanism of DNA damage caused by these agents and resulted in synergistic cell killing as measured by induction of NAP and Caspase 3 cleavage. In vitro, selinexor (5 mg/kg) and radiation (5 Gy) decreased total survival almost twice in AS94 (50 nM cell line) by 12% and 30% respectively, relative to vehicle whereas combination of selinexor and radiation resulted in 80% tumor destruction.

Conclusion: Selinexor inhibits the DNA repair mechanism in solid and hematological cancer cell lines and combination of selinexor with agents that cause DNA damage induces cancer cell death that is superior to each therapy alone. These data suggest that such a combination treatment is predicted to result in synergistic therapeutic outcome in cancer patients.

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Figure 2A: Immunohistochemistry images showing expression of DDR proteins in cell line AS94 (A) untreated (B) treated with selinexor 100 nM. selinexor down-regulated the expression of DDR genes in cell lines with lower sensitivity. In all cases, as the dose increased, the protein expression decreased.

Figure 3A: Immunohistochemistry analysis images showing expression of DDR proteins in cell line AS94 (A) untreated (B) treated with selinexor 100 nM. selinexor down-regulated the expression of DDR genes in cell lines with lower sensitivity. In all cases, as the dose increased, the protein expression decreased.

Figure 3B: Immunohistochemistry analysis images showing expression of DDR proteins in cell line AS94 (A) untreated (B) treated with selinexor 100 nM. selinexor down-regulated the expression of DDR genes in cell lines with lower sensitivity. In all cases, as the dose increased, the protein expression decreased.

Figure 4: Selinexor inhibited the expression of DDR proteins in cell lines with lower sensitivity. In all cases, as the dose increased, the protein expression decreased.

Figure 5: Immunofluorescence images showing expression of DDR proteins in cell line AS94 (A) untreated (B) treated with selinexor 100 nM. selinexor down-regulated the expression of DDR genes in cell lines with lower sensitivity. In all cases, as the dose increased, the protein expression decreased.

Selinexor Inhibits Recovery From Chemotherapeutic Agents Induced DNA Damage

Results: Treatment of solid and hematological cancer cell lines with selinexor did not induce DNA damage but increased the expression of DNA damage repair (DDR) proteins: MSH2, MLH1, PMS2, Rad51, and CtBP1. Selinexor regulates the expression of Chkl, Rad51, MSH6, MSH3 and MLH1 on the transcriptional level and PMS2 expression on the posttranslational level. There was a trend between the degree of DNA damage-repair-proteins reduction in sensitivity. Knock down of Chkl alone, induced cytoxicity whereas silencing of the other DDR proteins did not affect cell viability. Selinexor treatment following exposure to the DNA damaging agents decreased and silenced the repair mechanism of DNA damage caused by these agents and resulted in synergistic cell killing as measured by induction of NAP and Caspase 3 cleavage. In vitro, selinexor (5 mg/kg) and radiation (5 Gy) decreased total survival almost twice in AS94 (50 nM cell line) by 12% and 30% respectively, relative to vehicle whereas combination of selinexor and radiation resulted in 80% tumor destruction.

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Selinexor Regulates Expression of DDR Genes on the RNA and Protein Levels

Figure 6: Selinexor inhibited the expression of DDR proteins in cell lines with lower sensitivity. In all cases, as the dose increased, the protein expression decreased.

Figure 7: Selinexor inhibited the expression of DDR proteins in cell lines with lower sensitivity. In all cases, as the dose increased, the protein expression decreased.

Figure 8: Selinexor inhibited the expression of DDR proteins in cell lines with lower sensitivity. In all cases, as the dose increased, the protein expression decreased.

Figure 9: Selinexor inhibited the expression of DDR proteins in cell lines with lower sensitivity. In all cases, as the dose increased, the protein expression decreased.

Combination Treatment of Selinexor with Radiation shows Synergistic Effects in Lung Cancer Xenograft Model

Results: Treatment of solid and hematological cancer cell lines with selinexor did not induce DNA damage but increased the expression of DNA damage repair (DDR) proteins: MSH2, MLH1, PMS2, Rad51, and CtBP1. Selinexor regulates the expression of Chkl, Rad51, MSH6, MSH3 and MLH1 on the transcriptional level and PMS2 expression on the posttranslational level. There was a trend between the degree of DNA damage-repair-proteins reduction in sensitivity. Knock down of Chkl alone, induced cytoxicity whereas silencing of the other DDR proteins did not affect cell viability. Selinexor treatment following exposure to the DNA damaging agents decreased and silenced the repair mechanism of DNA damage caused by these agents and resulted in synergistic cell killing as measured by induction of NAP and Caspase 3 cleavage. In vitro, selinexor (5 mg/kg) and radiation (5 Gy) decreased total survival almost twice in AS94 (50 nM cell line) by 12% and 30% respectively, relative to vehicle whereas combination of selinexor and radiation resulted in 80% tumor destruction.

Conclusion: Selinexor inhibits the DNA repair mechanism in solid and hematological cancer cell lines and combination of selinexor with agents that cause DNA damage induces cancer cell death that is superior to each therapy alone. These data suggest that such a combination treatment is predicted to result in synergistic therapeutic outcome in cancer patients.

Summary of Results and Conclusions

- Selinexor treatment down-regulated the expression of DDR genes in the transcriptional and post-translational levels.
- Selinexor down-regulated DDR in both solid and hematological tumor cell lines and the effects were dose-dependent and sensitiviy to Sensitiviy.
- Inhibition of the DDR gene Chkl is toxic for cancer cells and selinexor drug combination with Chkl inhibitors showed additive cytocidal effects.
- Selinexor treatment did not induce DNA damage, but inhibited cell recovery from DNA damage leading to increased cell death.
- Pre-treatment with DNA damage inducing agents followed by exposure to selinexor was more toxic than the reversed sequential treatment with the two drugs.
- Combination treatment in combination with radiation showed synergistic anti-tumor activity in a NSCLC xenograft mouse model.
- The combination treatment of selinexor with DNA damage-inducing treatment is predicted to result in improved therapeutic outcome in cancer patients.