Activated Transcription Factor 3 (ATF-3) Expression is a Potential Marker of Tumor Response to the HDAC Inhibitor Pracinostat

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Background

Bladder cancer is the fourth most frequent cancer in men and sixth most common cancer affecting the Western world. About 50% of bladder cancer is superficial and the remaining 10% is muscle invasive. Surgery followed by platinum based chemotherapy is the standard treatment for high grade tumors, and results in <50% 5 year survival rate following surgery.

ATF3 can play a key role as either an oncogenic suppressor or an inducer in solid tumor depending on the degree of malignancy and cellular context. Decreased ATF3 expression in bladder cancer has been associated with tumor progression and a reduced survival rate (Yuan et al 2013).

Pracinostat (SB939) is an orally available highly potent histone deacetylase inhibitor (HDACi) with a sustained inhibitory effect on tumor issues (Novotny- Diemary et al 2010).

Aim

This study aimed to determine whether ATF3 expression which is silenced in bladder cancer could be induced by Pracinostat and used as a potential marker of response in HDACi mediated cancer therapy.

1 Pracinostat Treatment Reactivates ATF3 Expression in Bladder Cancer Cells

Effect of Pracinostat on human bladder cancer cells. (A) Western blot analysis of TSU-Prl cells cultured for 12hrs and 24hrs in the presence of increasing concentration of Pracinostat. ATF3 is reactivated in cells as dose and time increases. Progressive increase in histone proteins and non-histone protein acetylation with increasing concentration of Pracinostat and time. (B) Denaturation analysis of nuclear ATF3 protein expression upon treatment with Pracinostat.

Figure 1: Effect of Pracinostat on human bladder cancer cells. (A) Western blot analysis of TSU-Prl cells cultured for 12hrs and 24hrs in the presence of increasing concentration of Pracinostat. ATF3 is reactivated in cells as dose and time increases. Progressive increase in histone proteins and non-histone protein acetylation with increasing concentration of Pracinostat and time. (B) Denaturation analysis of nuclear ATF3 protein expression upon treatment with Pracinostat.

2 Pracinostat Treatment Inhibits Viability and Clonogenicity of Bladder Cancer Cells In Vitro and Tumor Growth In vivo

Figure 2: Reduced viability and clonogenicity of bladder cancer cells with extended treatment of Pracinostat. (A) Assessment of viability by viability assay demonstrated a dose dependent growth inhibition of TSU-Prl cells over a period of 5 days. (B) Increased proliferation of bladder cancer cells with depleted ATF3 on Pracinostat treatment. Viability assay on TSU-Prl cells transfected with scr (control) shRNA or with shRNA to knockout ATF3 expression treated with 200nM Pracinostat for 6 days. (C) Increased platinum responsive cell death is also observed in TSU-Prl cells treated with 500nM Pracinostat for 2 days. (D) Soft agar colony formation assay on TSU-Prl cells - Control, 200nM, and 500nM. (B) Colony number is reduced by ~75% (p = 0.006) in cells treated with 200nM Pracinostat and ~87% (p = 0.005) in cells treated with 500nM Pracinostat for 3 days (n=20). (F) Percentage body weight of Balb/c nude mice injected with 1x10^6 TSU-Prl cells during the period of treatment with Pracinostat (100mg/Kg) or Vehicle control (Mean ± SEM). (G) Tumor volume of mice treated Pracinostat (100mg/Kg) or Vehicle control (0.5% methylcellulose). 0.5/injection/5 days on - 2 days off orally (Mean ± SEM).

Figure 3: Pracinostat treatment reduces reactivation of ATF3 in xenograft tumor samples. Immuno - histological analysis of ATF3 expression (AC and H571-157) in serial sections of Pracinostat treated mice (A) Abd received 7 doses) and (B-E: 18 dose) of 100mg/Kg Pracinostat or in Vehicle control mice(C/F). ATF3 (yellow arrows) expression was persistent during the treatment in responder area (inner core) of Pracinostat treated mice xenografts and was undetectable in vehicle control mice. Ki67 expression (red arrow) was observed in abundance in vehicle control group. Ki67 expressing cells (periphery of tumor) in Pracinostat treated mice were different from cells expressing ATF3. A,B,D,E magnification X40 scale bar 50µm and C, F magnification X20 scale bar 100µm.

Figure 3: Pracinostat treatment reduces reactivation of ATF3 in xenograft tumor samples. Immuno - histological analysis of ATF3 expression (AC and H571-157) in serial sections of Pracinostat treated mice (A) Abd received 7 doses) and (B-E: 18 dose) of 100mg/Kg Pracinostat or in Vehicle control mice(C/F). ATF3 (yellow arrows) expression was persistent during the treatment in responder area (inner core) of Pracinostat treated mice xenografts and was undetectable in vehicle control mice. Ki67 expression (red arrow) was observed in abundance in vehicle control group. Ki67 expressing cells (periphery of tumor) in Pracinostat treated mice were different from cells expressing ATF3. A,B,D,E magnification X40 scale bar 50µm and C, F magnification X20 scale bar 100µm.

SUMMARY

• ATF3 expression is reactivated in bladder cancer cells treated with Pracinostat in vitro
• Pracinostat inhibits bladder cancer cell growth in vitro and in vivo
• Pracinostat reactivation of ATF3 expression correlates with tumor response in xenografts
• Pracinostat treatment induces cell cycle arrest in G1/G0 phase of bladder cancer cells activating RB1 (data not shown).

References


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