

Targeting Nrf2 with small molecules in human cancer cells

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Introduction

The transcription factor Nrf2 (nuclear factor erythroid 2-related factor 2) acts as a major sensor of oxidative stress and induces anti-inflammatory and chemopreventive effects. In the presence of oxidative stress, Nrf2 activates numerous cytoprotective and antioxidant genes, including NQO1 (NAD(P)H dehydrogenase (quinone 1)), a Phase II detoxification enzyme.

In cancer, the cytoprotective effects of Nrf2 assist in preventing carcinogenesis. However, there also exists a “dark side” of Nrf2 in cancer, with aberrant activation of Nrf2 associated with tumour promotion and chemoresistance¹. Nrf2 is overexpressed in several cancer types, including lung, pancreatic, breast, bladder, ovarian and melanoma, and is associated with a poor prognosis². Nrf2 inhibitors may therefore be useful in chemotherapy.

The aim of this study was to compare the effects of a range of small molecules reported to inhibit Nrf2³ across a variety of human cancer cell lines and validate their effects on Nrf2 and NQO1 expression in lung and melanoma cancer cells.

Methods

Cell viability: A549, H292, (lung) MiaPaCa2, BxPC3 (pancreatic), M202, A375 (melanoma) and MCF-7 (breast) human cancer cells were treated with Nrf2 inhibitors (brusatol, luteolin, retinoic acid (RA), ML385, triptolide, trigonelline hydrochloride and clobetasol propionate) for 72h and effects on proliferation measured by MTS assay.

Western blotting: Cells were stimulated for 4 h with inhibitors or dimethyl fumarate (DMF). Total cell lysates were then prepared and separated via SDS-PAGE. Nrf2 expression was measured by western blot analysis.

NQO1 activity: NQO1 activity was determined using an enzymatic assay⁴, following cell stimulation with Nrf2 inhibitors for 20h.

References

- 1- Wang X et al (2008). *Carcinogen*, 29, 1235-1243.
- 2- Kitamura H and Motohashi H (2018). *Cancer Sci*, 109, 900-911.
- 3- Zhu J et al (2016). *Free Rad Biol Med*, 99, 544-556.
- 4- Prochaska H and Santamaria A (1988). *Anal Biochem*, 169, 328-336.

Results

Table 1. IC₅₀ values of Nrf2 inhibitors in lung, pancreatic, melanoma and breast cancer cell lines.

Inhibitor	Mean IC ₅₀ (μM) values (±SEM)						
	A549	H292	MiaPaCa2	BxPC3	M202	A375	MCF-7
Brusatol	0.06 (±0.025)	0.015 (±0.006)	0.045 (±0.013)	0.028 (±0.003)	0.12 (±0.008)	0.03 (±0.003)	0.046 (±10.7)
Luteolin	122.37 (±37.09)	13.67 (±4.04)	41 (±15.6)	17.33 (±1.53)	23.46 (±5.28)	28.15 (±5.24)	9.31 (±1.6)
Retinoic acid (RA)	33.73 (±26.98)	82 (±53.02)	69 (±9.89)	>500	35.29 (±5.14)	40.09 (±6.08)	142.0 (±31.9)
ML385	>500	>500	>500	>500	>500	>500	>500
Triptolide	0.053 (±0.064)	0.019 (±0.008)	0.0178 (±0.007)	0.025 (±0.025)	0.028 (±0.009)	-	-
Trigonelline hydrochloride	>100	>100	>100	>100	>500	>500	>500
Clobetasol propionate	>100	>100	>100	>100	>100	-	-

1. Antiproliferative effects of Nrf2 inhibitors

Table 1 shows brusatol and triptolide to have the most potent antiproliferative effects in all cell lines (IC₅₀ values: 3-120nM and 17.8-53nM, respectively). Luteolin and retinoic acid varied in their anti-proliferative effects across different cell lines. ML385, trigonelline hydrochloride and clobetasol propionate showed no inhibitory effects at concentrations of >100μM.

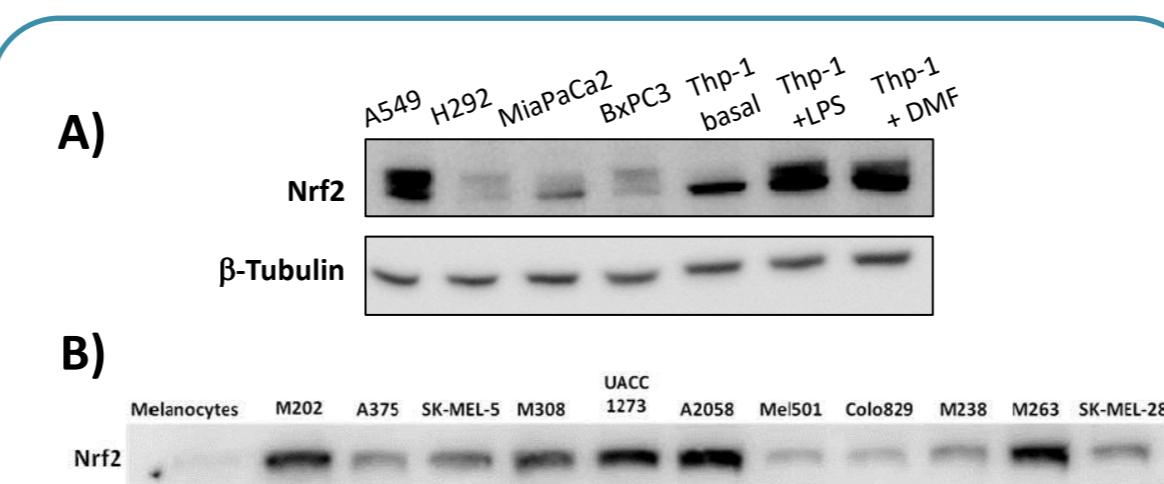


Figure 1. Nrf2 protein expression in lung, pancreatic and monocytic leukemia cells (A) and melanoma cells (B).

3. Effect of Nrf2 inhibitors on Nrf2 expression

Figure 2A and 2B show that triptolide and brusatol had the greatest inhibitory effects on Nrf2 expression in A549 and M202 cells when compared with vehicle control. Luteolin also partially reduced expression in M202 cells (Figure 2B). Trigonelline hydrochloride and RA did not appear to affect Nrf2 levels.

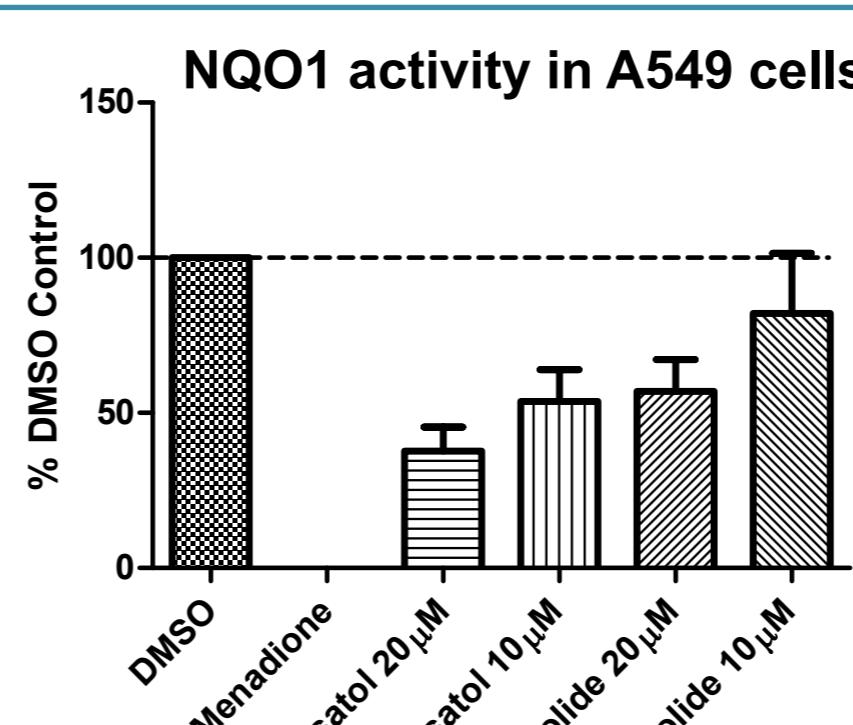


Figure 3. NQO1 activity in A549 cells following treatment with brusatol and triptolide for 20h.

2. Nrf2 expression in cancer cell lines

Figure 1A shows that A549 lung cells had the highest basal Nrf2 expression, similar to that of THP-1 cells activated with dimethylfumarate (DMF). In a range of human melanoma cells, M202, UACC 1273, A2058 and M263 showed the highest Nrf2 expression (Figure 1B). A549 and M202 cells were therefore taken forward for further experiments.

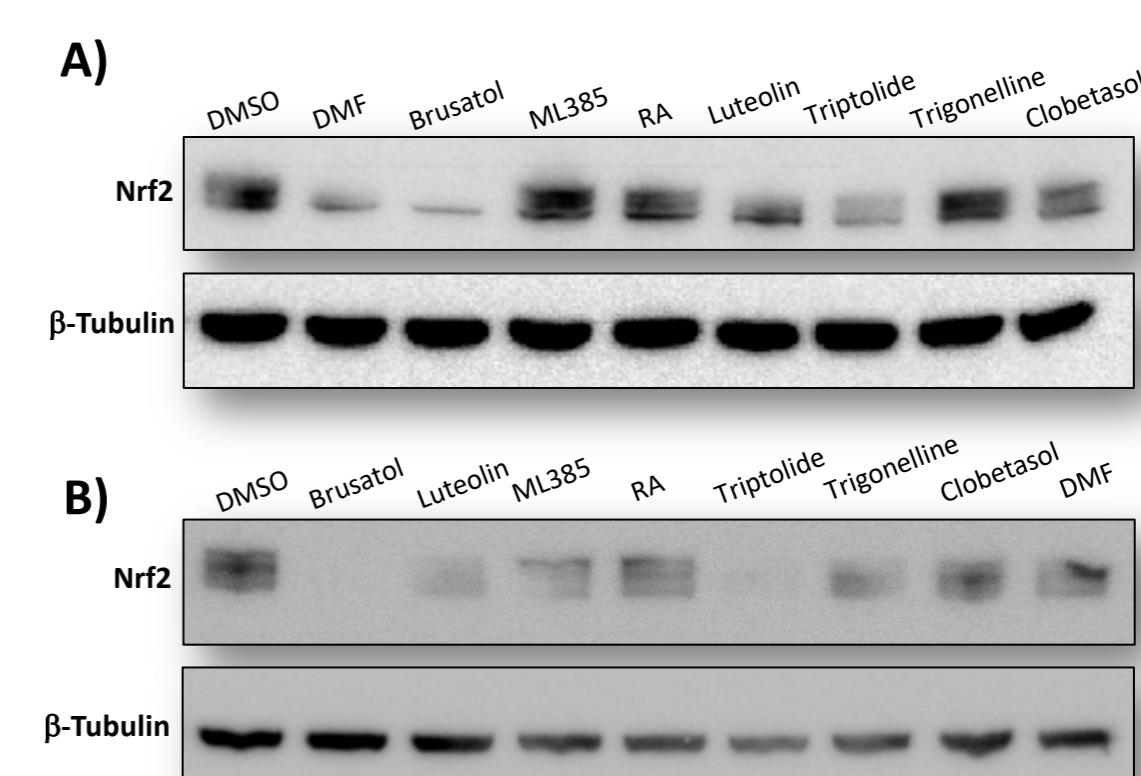


Figure 2. Nrf2 protein expression in A549 (A) and M202 (B) cells stimulated with Nrf2 inhibitors for 4h.

4. Effect of Nrf2 inhibitors on NQO1 activity in A549 cells

Figure 3 shows that brusatol reduced NQO1 activity when compared with control. Triptolide also reduced NQO1 activity, however this reduction was not as dramatic as that observed with brusatol.

Conclusion

- Brusatol and triptolide were the most potent inhibitors of cell proliferation in all cell lines and reduced Nrf2 protein levels and NQO1 activity.
- Brusatol has off-target effects; however, triptolide, despite potential toxicity, may have potential benefit as a targeted therapy in cancers overexpressing Nrf2.
- ML385 and trigonelline hydrochloride had no effects on Nrf2 expression or cell proliferation, possibly due to reduced cell uptake.