Inhibition of human FAHNSCC OHSU-974 cell line in vivo and in vitro by a nutrient mixture (PB) of quercetin, curcumin, green tea, cruciferex and resveratrol

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1. Objective:

Fanconi anemia (FA) is an autosomal recessive disease characterized by bone marrow failure, congenital abnormalities, chromosomal instability and cancer predisposition. Head and neck squamous cell carcinoma (HNSCC) and acute myeloid leukemia are the major causes of mortality and morbidity in FA patients. The objective of this study was to investigate the antineoplastic activity of a specific nutrient mixture (PB) of quercetin, curcumin, green tea, cruciferex and resveratrol on OHSU-974 human cell line FAHNSCC in vivo and in vitro.

2. Methods and Materials:

A. Human FAHNSCC cell line OHSU-974 (Fanconi Anemia Research Fund, Oregon Health & Science University, Portland, Oregon, USA) was cultured in RPMI medium supplemented with 20% FBS and antibiotics. At near confluence, cells were treated in triplicate with different concentrations of PB: 0, 10, 25, 50, 75 and 100 µg/ml. Cells were also treated with PMA (100 ng/ml) to induce MMP-9 activity. Cell proliferation was detected by MTT assay, secretion of MMPs by gelatinase zymography, invasion through Matrigel, migration by scratch test and morphology by H&E staining.

B. In vivo, athymic male nude mice (n=12) were inoculated with $3x10^6$ OHSU-974 cells subcutaneously and randomly divided into two groups: group A was fed a regular diet and group B a regular diet supplemented with 1% PB. Four weeks later, the mice were sacrificed and their tumors were excised, weighed and processed for histology.

Compostion of Phytobiologicals (PB)

Nutrient	Proportion
Quercetin	400mg
Cruciferex	400mg
Curcumin	300 mg
Standardized Green Tea Extract (80% polyphenol)	300 mg
Resveratrol	50 mg

3. Results:

1. PB inhibited the growth of head and neck squamous carcinoma cell line of Fanconi Anemia patient OHSU-974 tumor by 67.6% ($p<0.0001$) and tumor burden by 63.6% ($p<0.0001$). See Figures 1A, 1B.

Figure 1A - Effect of PB on tumor weight of OHSU-974 xenograft

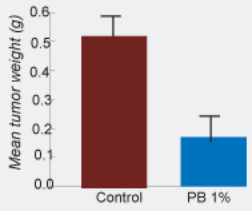
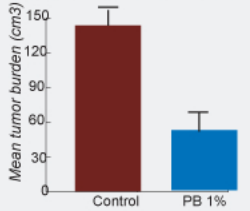
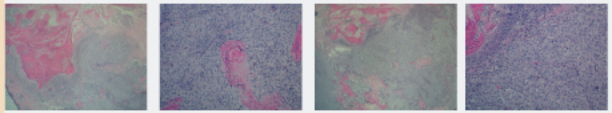


Figure 1B - Effect of PB on tumor burden of OHSU-974 xenograft



2. The tumors in both groups were shown to be irregularly round, ulcerated, subcutaeous masses consistent with squamous cell carcinoma, as shown in Figure 2. The PB group tumors were smaller than the control tumors.

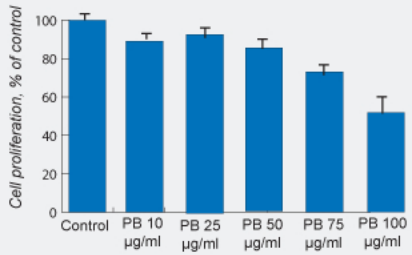
Figure 2 - Histopathology of representative tumors from groups.



2A-Control 40x 2B-Control 200x 2C-PB 1% 40x 2D-PB 1% 200x

3. PB inhibited growth of OHSU-974 by 10% ($p=0.01$), 25% ($p=0.0003$) and 50% ($p=0.0004$) at 50, 75 and 100 µg/ml (Figure 3).

Figure 3 - Effect of PB on head & neck squamous carcinoma cell line of fanconi anemia patient OHSU-974: MTT 24h



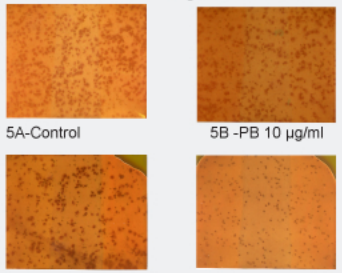
4. Zymography revealed MMP-2 and PMA-induced MMP-9 secretion. PB suppressed OHSU-974 cell secretion of both MMPS at 10 and 25 µg/ml, with total block at 50 µg/ml (Figure 4).

Figure 4- Zymograms of untreated (4A) and PMA-treated (4B) cells

Legend: 1 - Markers, 2- Control, 3-7 - PB 10, 25, 50, 75, 100 µg/ml

5. PB inhibited OHSU-974 invasion through Matrigel in a dose-dependent fashion with total block at 50 µg/ml. Photomicrographs shown in 5A-5F.

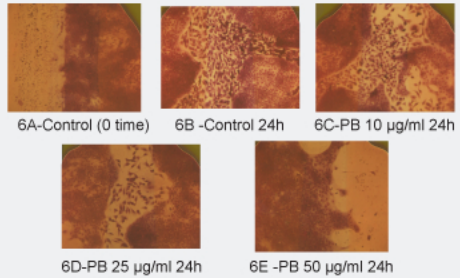
Figure 5 - Effect of NM on Matrigel invasion of OHSU-974 cells



5A-Control 5B -PB 10 µg/ml 5C-PB 25 µg/ml 5D -NM 50µg/ml

6. PB inhibited OHSU-974 migration (scratch test) in a dose-dependent fashion with total block at 50 µg/ml (Figure 6).

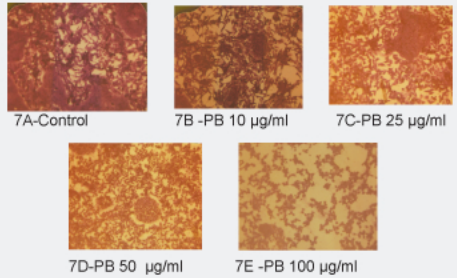
Figure 6 - Effect of PB on OHSU-974 cell migration



6A-Control (0 time) 6B -Control 24h 6C-PB 10 µg/ml 24h 6D-PB 25 µg/ml 24h 6E -PB 50 µg/ml 24h

3.7 H&E staining showed no morphological changes below 50 µg/ml (Figures 7A-F).

Figure 7 - Effect of NM on OHSU-974 cell morphology: H&E



7A-Control 7B -PB 10 µg/ml 7C-PB 25 µg/ml 7D-PB 50 µg/ml 7E -PB 100 µg/ml

4. Conclusion:

The results suggest that PB has potential therapeutic use in the treatment of human FA HNSCC.

5. Translational Applicability:

Current treatment methods for Fanconi Anemia-associated cancers are generally ineffective. Thus, there is a need for development of effective therapeutic agents for these cancers with minimal toxicity. Our results demonstrate that PB significantly inhibited the growth and tumor burden of FA HNSCC cell line OHSU-974 in vivo. In addition, invasive parameters, such as MMP-2 and -9 secretion and invasion were significantly inhibited by PB in vitro. These findings suggest the potential of PB in FA HNSCC treatment.