

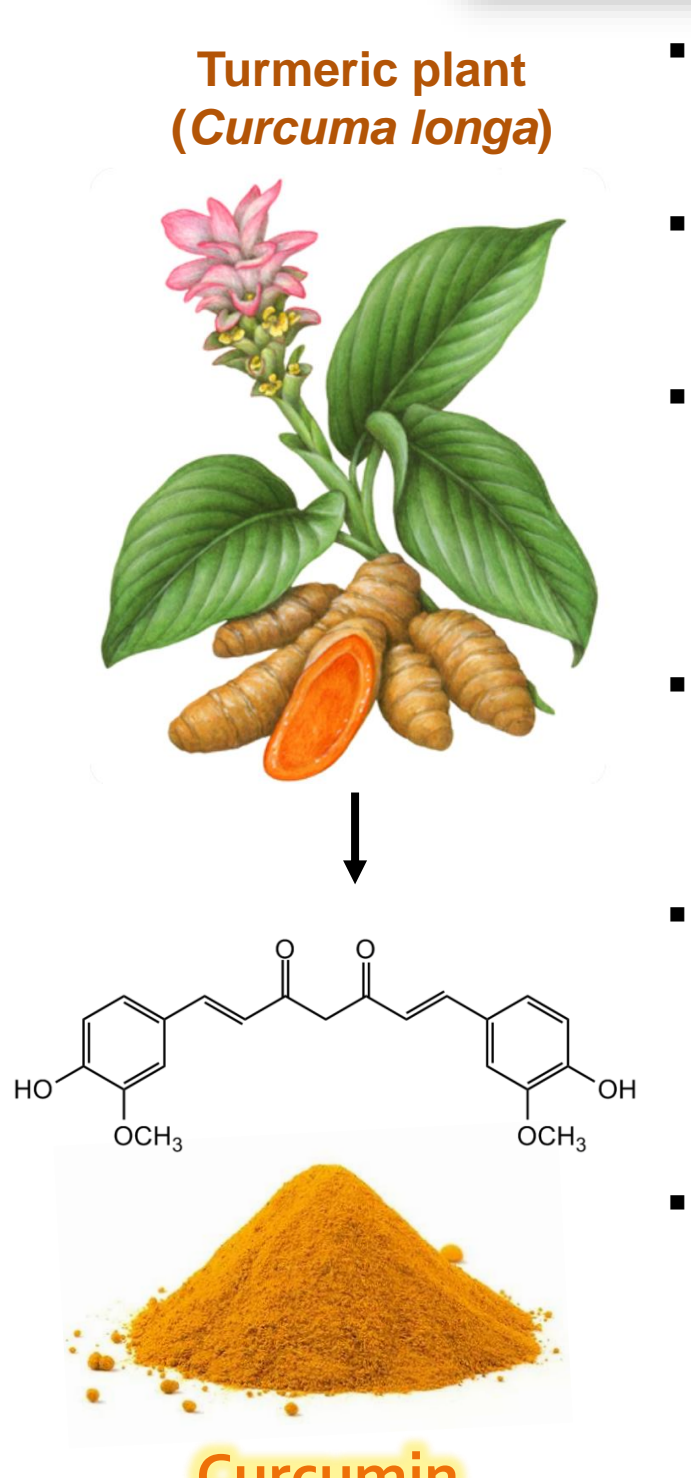
Curcumin modulates cell type-specific miRNA networks to induce cytotoxicity in ovarian cancer cells

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Abstract

Curcumin, the natural polyphenolic compound extracted from the spice *Curcuma longa*, has proven anti-cancer activity against various human cancers. It exerts anti-tumorigenic activity by regulating multiple molecular targets and signaling cascades. Upstream, it can regulate non-coding RNAs like miRNAs and is an epigenetic modulator. An mRNA-miRNA integration study was performed to unravel the deregulated miRNAs involved in curcumin-induced cytotoxicity in two ovarian cancer cell lines: PA1 and A2780. The bioinformatic analysis and the experimental validations revealed that curcumin activity is very cell-type specific as distinct miRNAs modulated multiple modes of cellular cytotoxicities like apoptosis, autophagy, DNA damage responses, and impairment of stemness. In both curcumin-treated PA1 and A2780 cells, the expression of several miRNAs demonstrated an inverse correlation with their respective direct targets. The oncomiR/tumor-suppressor miRNA profiles revealed suppression of oncogenic processes by curcumin. Curcumin induced reversal of multiple cisplatin-resistance genes, suggesting a moderate cisplatin-sensitisation effect in both cells. Curcumin treatment also impaired epithelial-to-mesenchymal transition (EMT) characteristics like migratory and colony-forming capabilities in these cells. These findings further highlight the molecular mechanism of curcumin action in ovarian cancers.

Curcumin

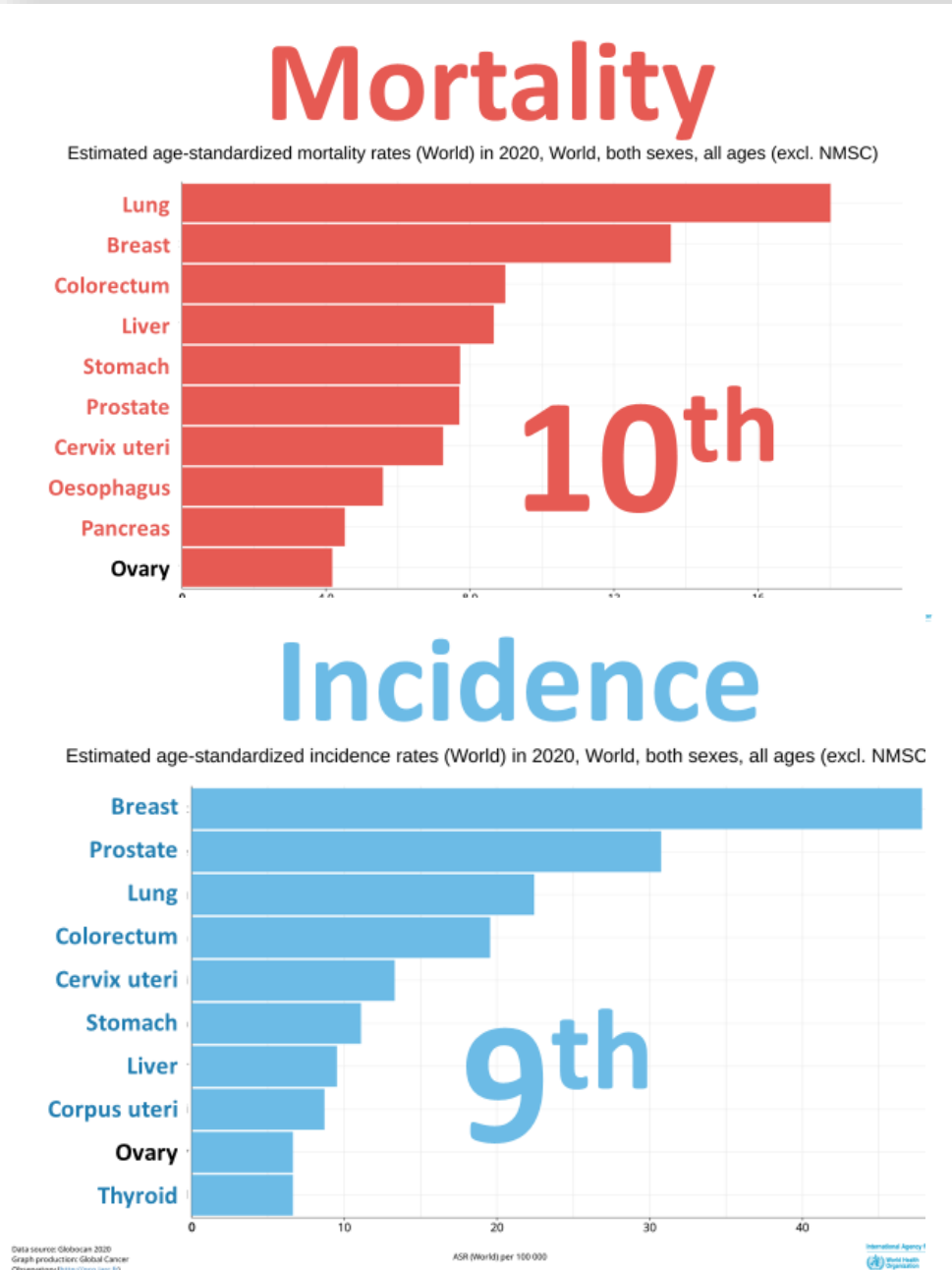


Turmeric plant (*Curcuma longa*)

Curcumin

- Curcumin has proven anti-cancer activity against various human cancers
- Curcumin targets only cancer cells, leaving healthy cells unaffected
- It has entered into Phase III clinical trials as monotherapy and in combination therapies for colon, prostate, and pancreatic cancers
- Curcumin reduced cancer therapy side effects like radiation induced dermatitis and oral mucositis
- Curcumin also exhibits antioxidant, anti-aging, anti-inflammatory, anti-microbial, anti-proliferative, and anti-angiogenic properties
- Curcumin exerts such diverse therapeutic properties by interacting and modulating multiple molecular targets, and signalling cascades
- Curcumin regulates non-coding RNAs and is an epigenetic modulator

Ovarian cancer

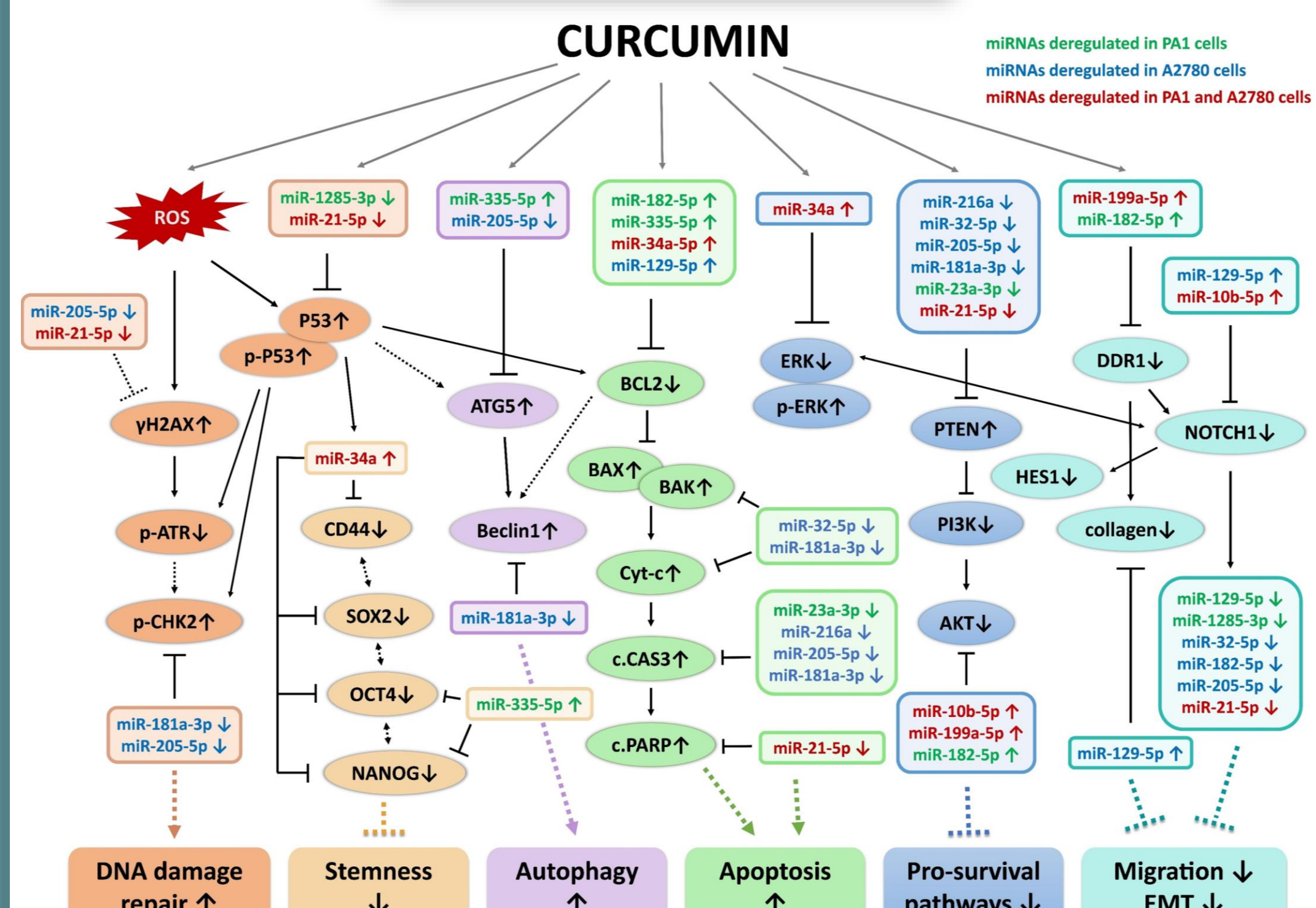


Mortality

Incidence

Ovarian cancer ranks 9th in incidence and 10th in mortality among all cancers worldwide. It is a fatal gynecological cancer, due to advanced stage diagnosis, high metastatic potential and recurrence due to its chemoresistant nature.

Conclusion



CURCUMIN

miRNAs deregulated in PA1 cells
miRNAs deregulated in A2780 cells
miRNAs deregulated in PA1 and A2780 cells

DNA damage repair ↑, Stemness ↓, Autophagy ↑, Apoptosis ↑, Pro-survival pathways ↓, Migration ↓, EMT ↓

RESULTS

mRNA-miRNA integration analysis in curcumin-treated PA1 and A2780 cells



A Cell viability assay of PA1 and A2780 cells treated with curcumin showing an IC50 of ~10 μM. **B** miRmapper bar plot representing dominant miRNAs affecting DEG targets in curcumin-treated PA1 and A2780 cells. **C** Venn diagram representing common and unique genes/miRNAs in curcumin-treated PA1 and A2780 cells. **D** Curcumin induced pathways deduced from miRNA-mRNA integrated analysis and from mRNA DEGs.

Curcumin-induced downregulation of survival pathways and activation DNA damage responses



A Western blot images and quantifications of signaling cascades in 10 μM curcumin-treated PA1 cells. **B,C** Western blot images and quantifications of DNA damage response protein expressions in 10 μM curcumin-treated PA1 cells.

Curcumin-induced reversal of TSGs/OGs expressions



Bar plot representing reversed expression of oncogenes and TSGs in curcumin-treated PA1 and A2780 cells compared to TCGA ovarian cancer dataset

Status of curcumin-induced genes in Indian ovarian cancer subtypes



Bar plot representing the expression profiles of validated gene expressions from the Indian ovarian cancer cohort

Curcumin-induced induction of apoptosis in PA1 cells



A Histograms and bar graph quantification of cell cycle assay in 10 μM curcumin-treated PA1 cells. **B** Dot plots and bar graph quantifications of apoptosis assay by Annexin V-FITC/PI double staining in 10 μM curcumin-treated PA1 cells. **C** Western blot images and quantifications of apoptotic/autophagic protein expressions in 10 μM curcumin-treated PA1 cells.

Curcumin-induced induction of apoptosis & autophagy in A2780 cells



A Histograms and bar graph quantification of cell cycle assay in 10 μM curcumin-treated A2780 cells. **B** Dot plots and bar graph quantifications of apoptosis assay by Annexin V-FITC/PI double staining in 10 μM curcumin-treated A2780 cells. **C** Western blot images and quantifications of apoptotic/autophagic protein expressions in 10 μM curcumin-treated A2780 cells.

Chemo-sensitising effect of curcumin



Bubble plot representing downregulated cisplatin and anti-resistance genes in curcumin-treated PA1 and A2780 cells, indicating a moderate cisplatin-sensitisation effect of curcumin

Curcumin-induced activation of ROS, downregulation of EMT characteristics and stemness potential



A Induction of ROS in 10 μM curcumin-treated PA1 and A2780 cells with/without NAC. **B** Wound healing assay images in curcumin-treated PA1 cells. **C** Colony formation assay images and quantification of colony area in curcumin-treated PA1 and A2780 cells. **D** Relative expression level of stemness genes in curcumin-treated PA1 and A2780.

Impact of curcumin on TSGs/OGs and TSmRs/OncomiRs



A Heatmap of upregulated TSGs and downregulated OGs in curcumin-treated PA1 and A2780 cells. **B** Bubble plot representing the upregulated TSmRs and downregulated oncomiRs in curcumin-treated PA1 and A2780 cells.

Induction of cell-type-specific miRNAs by curcumin



A Relative expression levels miRNAs in curcumin-treated PA1 and A2780 cells. **B** Western blot images and quantifications of DDR1 signaling cascades. **C** Luciferase assay depicting miR-199a-5p regulation of DDR1. **D** Curated regulatory network of the transcription factor, validated miRNAs, and validated protein targets from curcumin-treated PA1 and A2780 cells. **E** Curated regulatory network of the transcription factor, validated miRNAs, and validated protein targets from curcumin-treated PA1 and A2780 cells. **F** collagen expression upon curcumin treatment

Acknowledgements

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