Targeting the differentiation of gastric cancer cells (KATO-III) downregulates epithelial-mesenchymal and cancer stem cell markers

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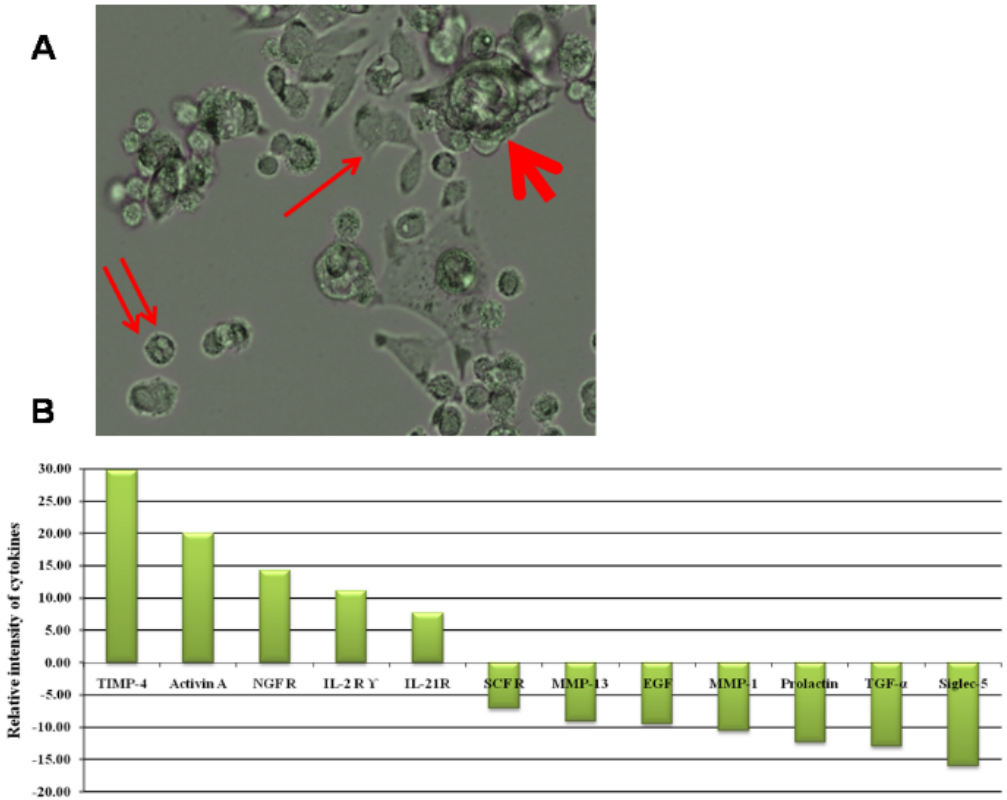
Abstract: The aim of the present study was to analyze the acquisition of the differentiated phenotype in the human gastric signet ring cell adenoma cancer KATO-III cell line *in vitro*. The morphology of KATO-III cells was explored by microcinematography. Different cytokines secreted by both adherent and non-adherent KATO-III cells into medium were observed. The cancer stem cell phenotypes were identified by reverse transcription-quantitative polymerase chain reaction using primers (E-Cad, Slug, Snail, vimentin, NANOG, NESTIN, OCT3/4 and C-X-C motif chemokine receptor 4) or antibodies [CD 90 and CD117] by flow cytometry (FACS). The influence of the induction media for the differentiation of mesenchymal cells was studied through viability and proliferation assays, by evaluating gene expression and the expression of markers via FACS. Cell viability and cell cycle distribution were evaluated following the treatment of KATO-III with acetyl salicylic acid and using the induction media as an inhibitor of epithelial-mesenchymal transition (EMT)and heparanase. A total of 3 phenotypes of KATO-III were observed (adherent, non-adherent and cell cluster), which have internal potential for cell transition into one of the other phenotypes. KATO-III was differentiated into adipocyte-, chondrocyte-, osteocyte- and neurocyte-like cells by the induction media. Identification of the induced cells was conducted using cell dyes. Reduced mRNA expression of EMT-associated molecules, stem cell markers and heparanase was observed with acetyl salicylic acid and induction media. An inhibitory effect of acetyl salicylic acid and the induction media was also noted in regard to cell proliferation. In addition, acetyl salicylic acid induced G0/G1 phase cell cycle arrest in KATO-III cells.

1- Spheroid cluster formation and significantly different expression ratio of cytokines (adherent/non-adherent) in the KATO-III cell line



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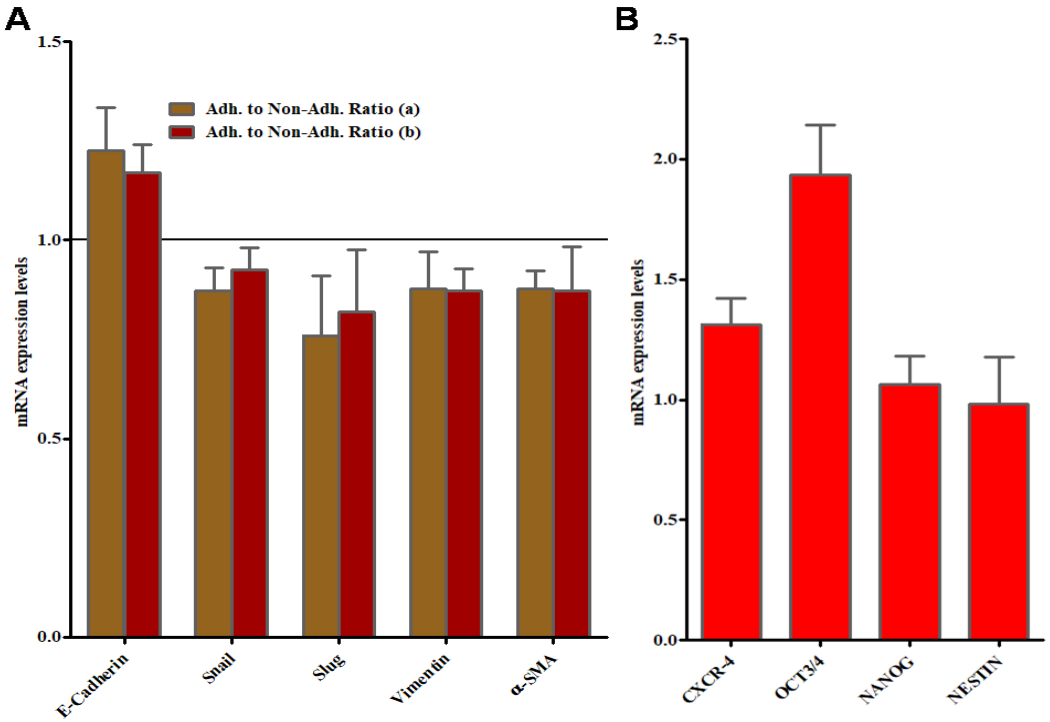
KATO-III cell line in vitro.

Spheroid cluster formation and significantly different expression ratio of cytokines (adherent/non-adherent) in the KATO-III cell line (fold-change >5; P<0.05).
(A)KATO-III cells form adherent (arrow), non-adherent (double arrow) and cell clusters (bold arrow; some were also non-adherent. (B)Of the 174 proteins proposed in this test only 12 cytokines were reported to be significant in the ratio of cytokine expression in adherent/non-adherent KATO-III cell line. P<0.05.

2- KATO-III cell line present cancer stem cell as well as epithelial mesenchymal transition markers.



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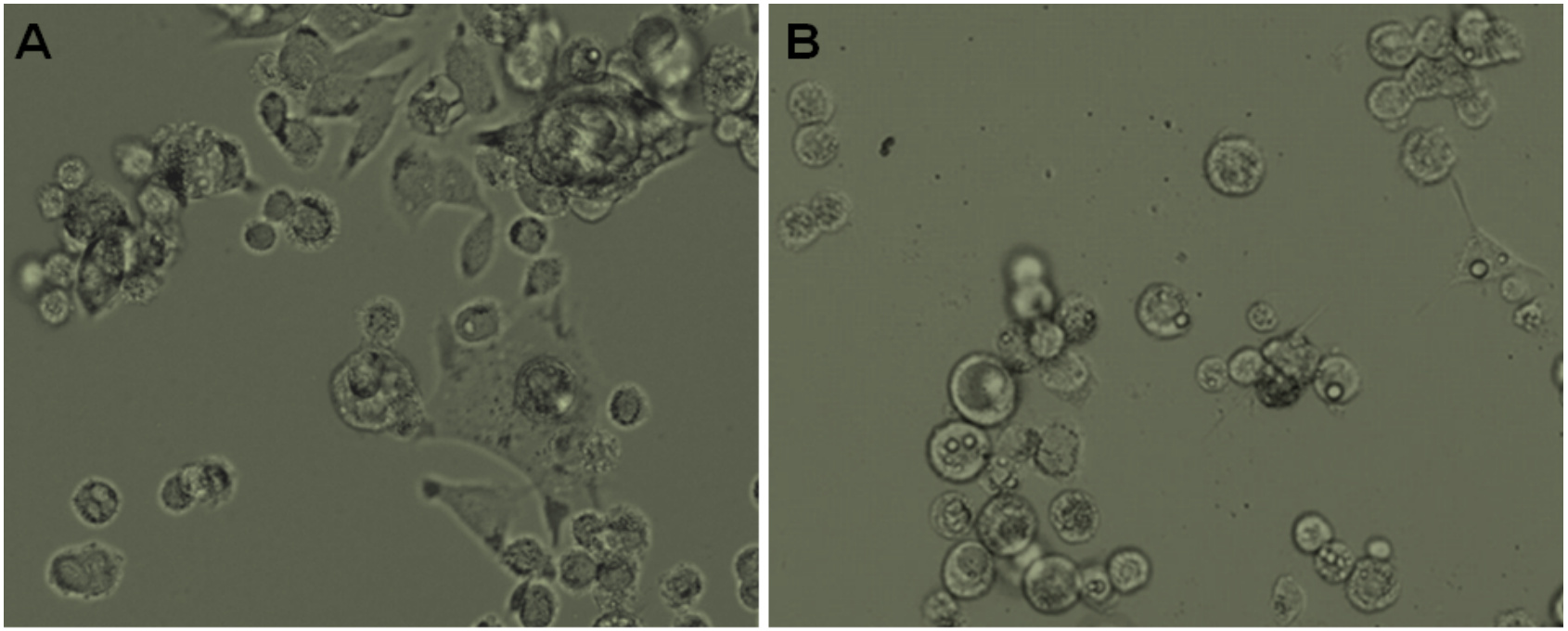
KATO-III cells present CSC as well as EMT markers.

mRNA gene expression of EMT-associated genes and stem cell markers in the KATO-III cell line. (A) RT-qPCR analysis revealed no alterations in the gene expression of EMT-associated molecules when adherent and non-adherent KATO-III cells were grown separately (a) prior to and (b) following 1 week ($P<0.05$).(B) mRNA gene expression of stem cell markers (CXCR-4, NANOG, OCT3/4 and NESTIN) in the KATO-III cell line via RT-qPCR. EMT, epithelial-mesenchymal transition; RT-qPCR, reverse transcription-quantitative polymerase chain reaction;CXCR-4, C-X-C chemokine receptor type 4;OCT3/4, octamer-binding transcription factor 3/4.

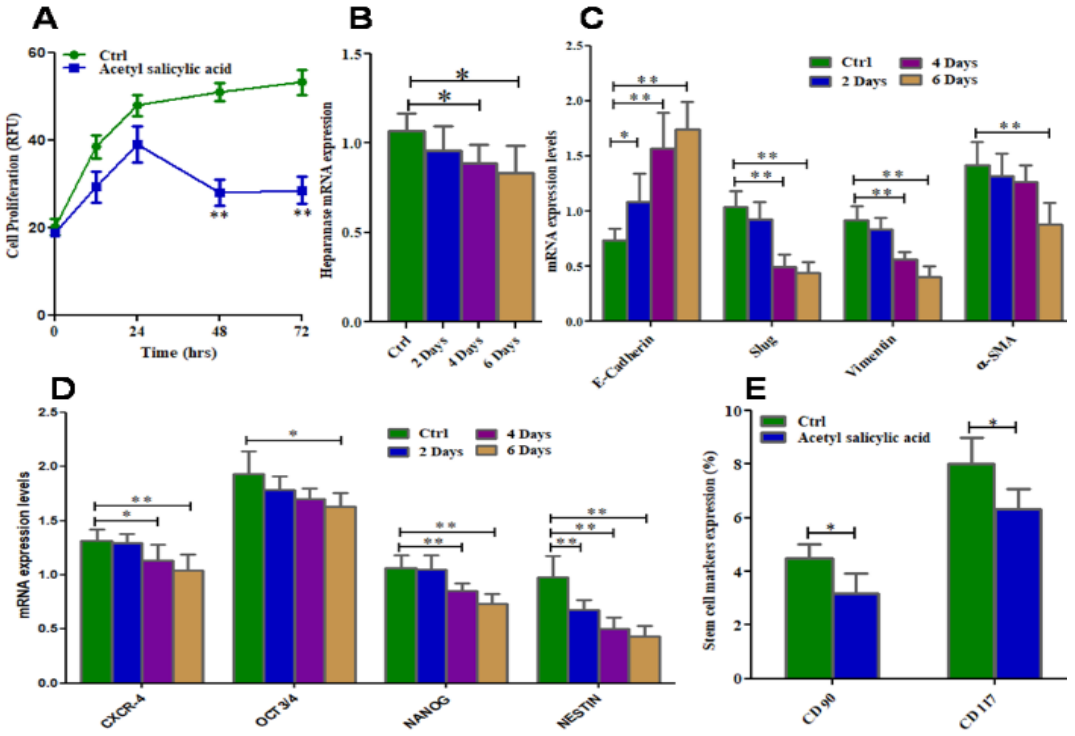
3- Acetyl salicylic acid modifies heparanase, EMT and CSC marker expression in KATO-III cells in vitro.



Effects of acetyl salicylic acid on the morphology of KATO-III cells

The phase–contrast images were captured at ×400-magnification 24 hrs before (A) and after (B) treatment with 4.5mM acetyl salicylic acid. At least three separate experiments were carried out with results similar to those shown here.

4- Acetyl Salicylic Acid modify heparanase expression and gastric cancer cell behavior *in vitro*.



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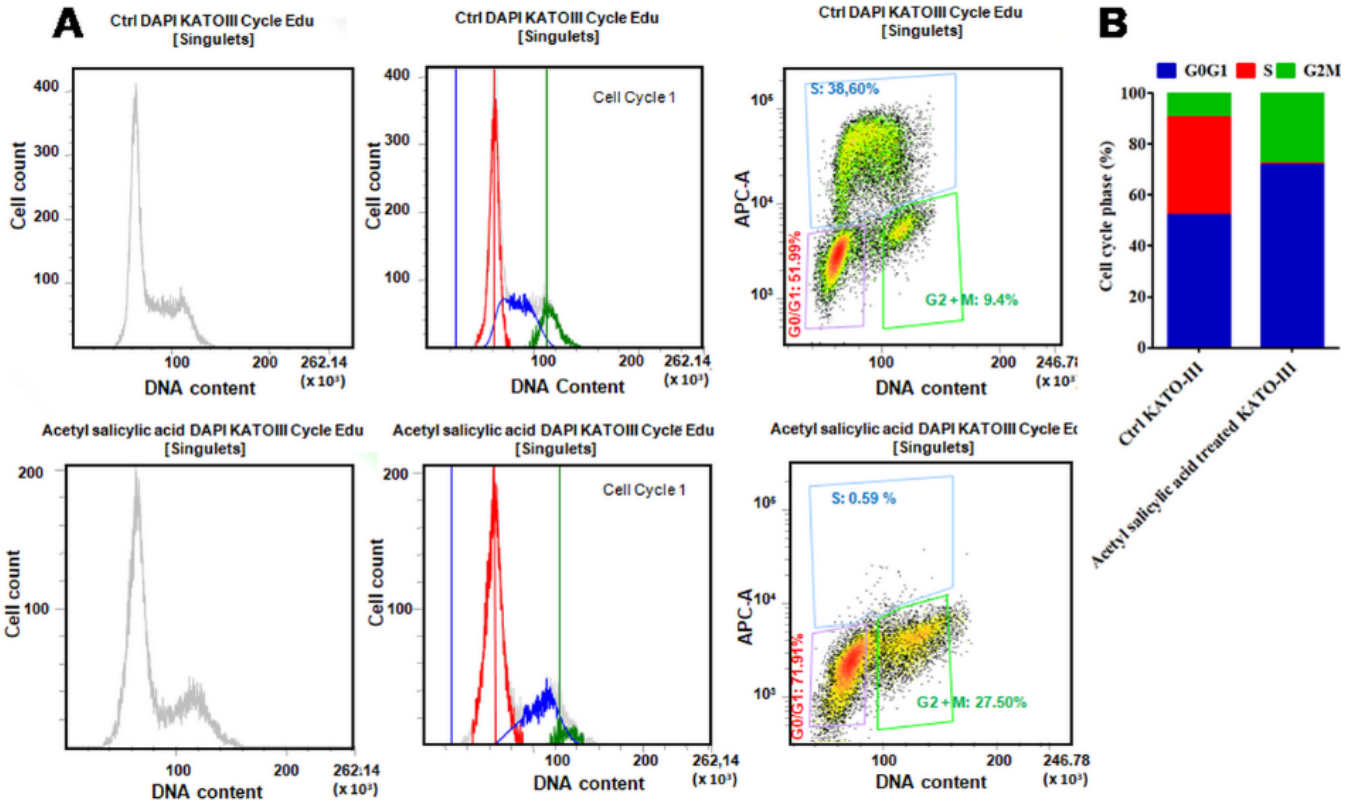
Acetyl salicylic acid modifies heparanase, EMT and CSC marker expression in KATO-III cells in vitro.

Cell proliferation as well as mRNA expression of EMT-associated genes and stem cell markers in KATO-III following treatment with 4.5mM acetyl salicylic acid for 6 days.(A) Cell proliferation is inhibited, and (B) the expression of heparanase, (C) mesenchymal markers (Slug, vimentin and α-SMA) and (D) stem cell markers CXCR-4, OCT3/4, NANOG and NESTIN as well as (E) CD90 and CD117 were lower, while those of (C) the epithelial marker E-cadherin was higher in KATO-III cells following treatment with acetyl salicylic acid as determined by reverse transcription-quantitative polymerase chain reaction and flow cytometry. (*P<0.05 and **P<0.01). EMT, epithelial-mesenchymal transition; α-SMA, α-smooth muscle actin; CXCR-4, C-X-C chemokine receptor type 4; OCT3/4, octamer-binding transcription factor3/4; CD, cluster of differentiation

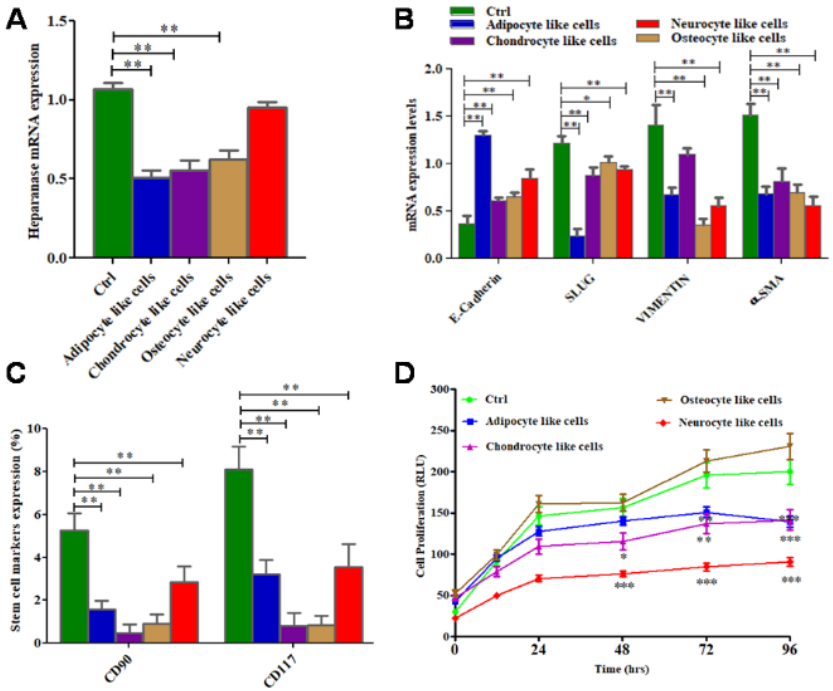
5- Acetyl Salicylic Acid modify cell cycle in cancer cells



Acetyl salicylic acid modifies the cell cycle in KATO-III cells.

Cell cycle arrest of the KATO-III cell line via flow cytometry following treatment with 4.5mMacetyl salicylic acid for 4days.KATO-III cells were analyzed by flow cytometry for cell cycle distribution following treatment with 4.5mM acetyl salicylic acid for 4days. (A) G0/G1, S and G2/M phase cells presented as percentages. (B)Data are expressed as the mean ± standard error of the mean of at least 3 independent experiments.

6- Cell inducer differentiation medium down heparanase and stem cell marker expression as well as cell proliferation



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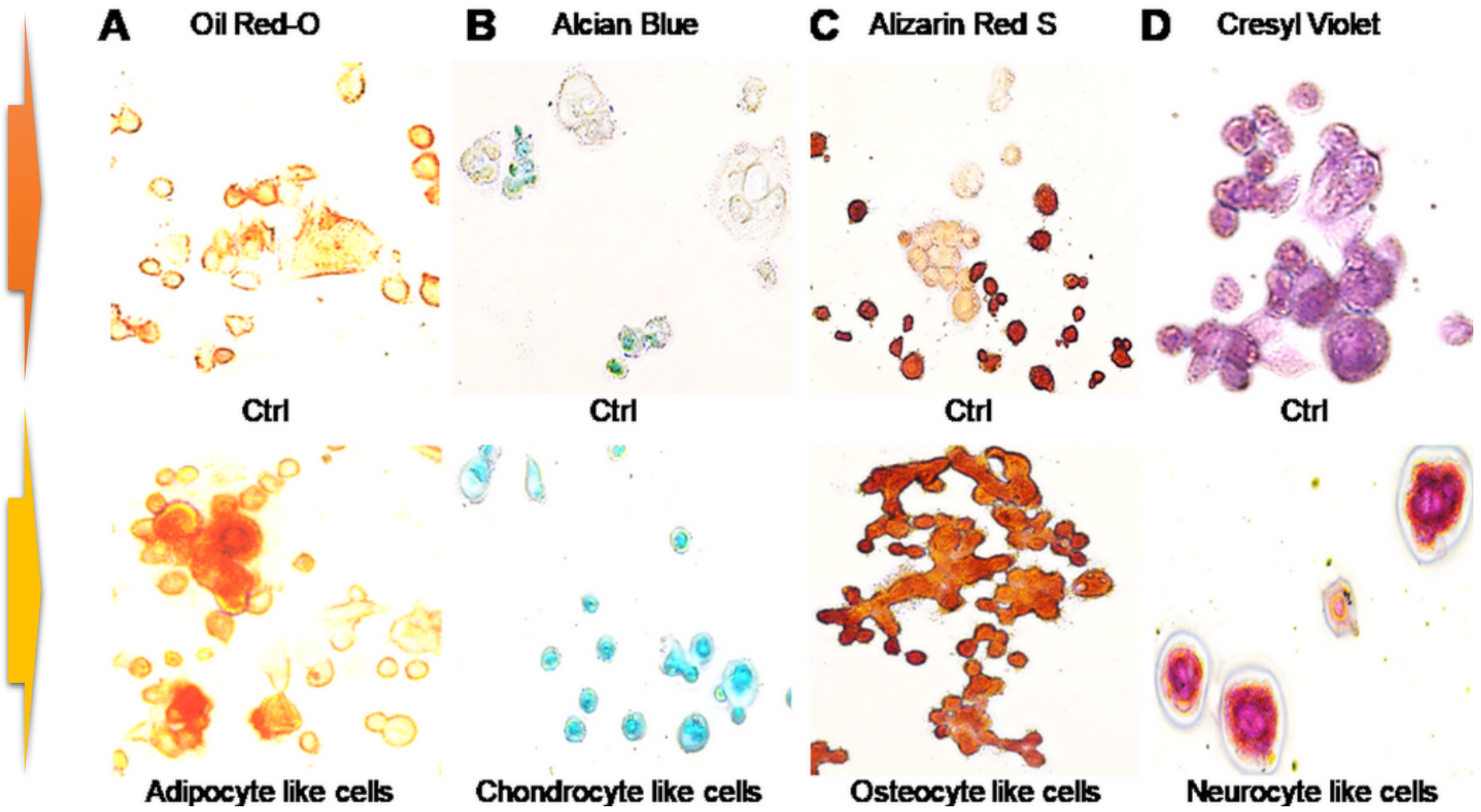
Cell inducer differentiation media downregulates heparanase and stem cell marker expression as well as the inhibition of cell proliferation.

mRNA expression of heparanase and EMT markers via reverse transcription-quantitative polymerase chain reaction and stem cell marker expression via flow cytometry as well as cell proliferation in differentiated KATO-III cells following induction. of(A) heparanase, (C) stem cell markers (CD90 and CD117)and(B)mesenchymal markers (Slug, Vimentin and α-SMA) were reduced while the epithelial marker (E-cadherin) was increased following the induction of differentiation. (D) Cell proliferation was also significantly inhibited. One- and two-way analysis of variance (ANOVA), followed by post-hoc Bonferroni's multiple comparison test for gene expression and cell proliferation respectively ($^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$). EMT, epithelial-mesenchymal transition; CD, cluster of differentiation; α-SMA, α-smooth muscle actin. ANOVA, analysis of variance.

7- Cell inducer differentiation medium induce cancer cell differentiation



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Cell-inducer differentiation medium induces cancer cell differentiation.

Coloration of differentiated KATO-III cells following induction.(A) Adipogenic, (B) chondrogenic, (C) osteogenic and (D) neurogenic differentiation of KATO-III cells as confirmed by coloration.

Conclusion

The induction of the differentiation of cancer stem cells into non-proliferating cells offers the possibility for novel drug design to overcome the issues associated with metastasis, drug resistance and systemic toxicity with improved therapeutic efficacy.