CHARACTERIZATION OF TUMOR INITIATING CELLS IN ESOPHAGEAL SQUAMOUS CELL CARCINOMA

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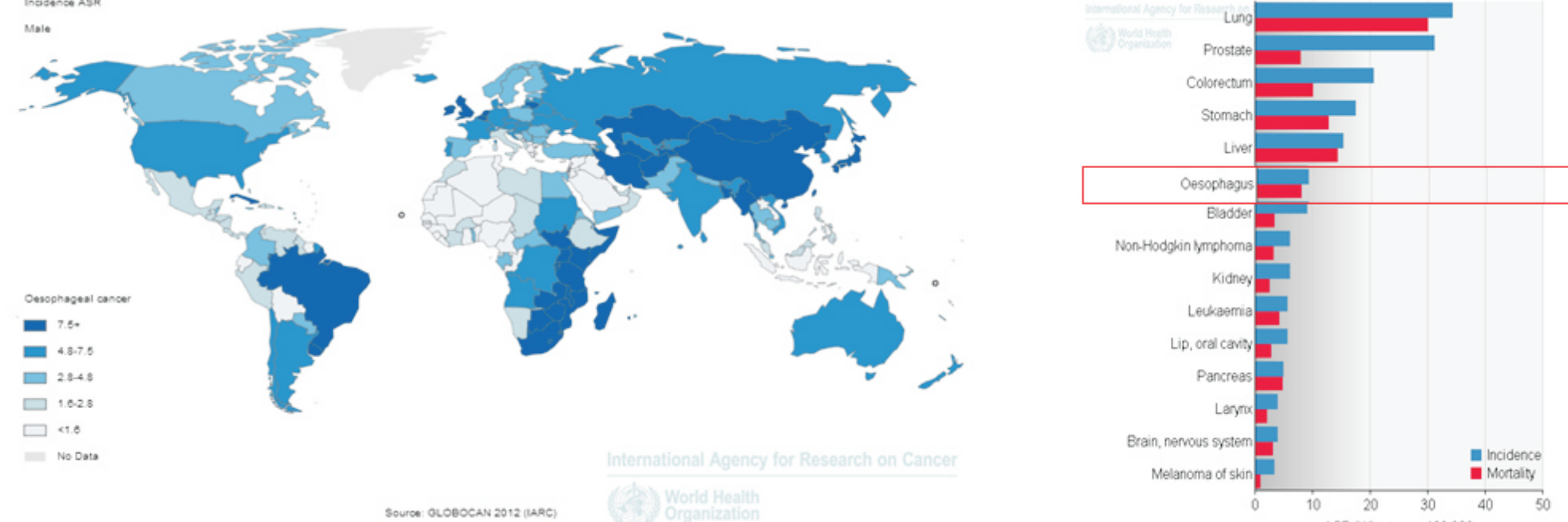
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INTRODUCTION

- Esophageal cancer is one of the most incident and lethal cancers in the world.

- Squamous cell carcinoma (ESCC) accounts for more than 80% of esophageal cancer cases.

No initial symptoms ➡ Late diagnosis ➡ Inefficient treatment

- Urgency for better understanding of the molecular mechanisms of ESCC development.
- Cancer Stem Cells Hypothesis can elucidate tumor heterogeneity and tumor recurrence after traditional therapy.
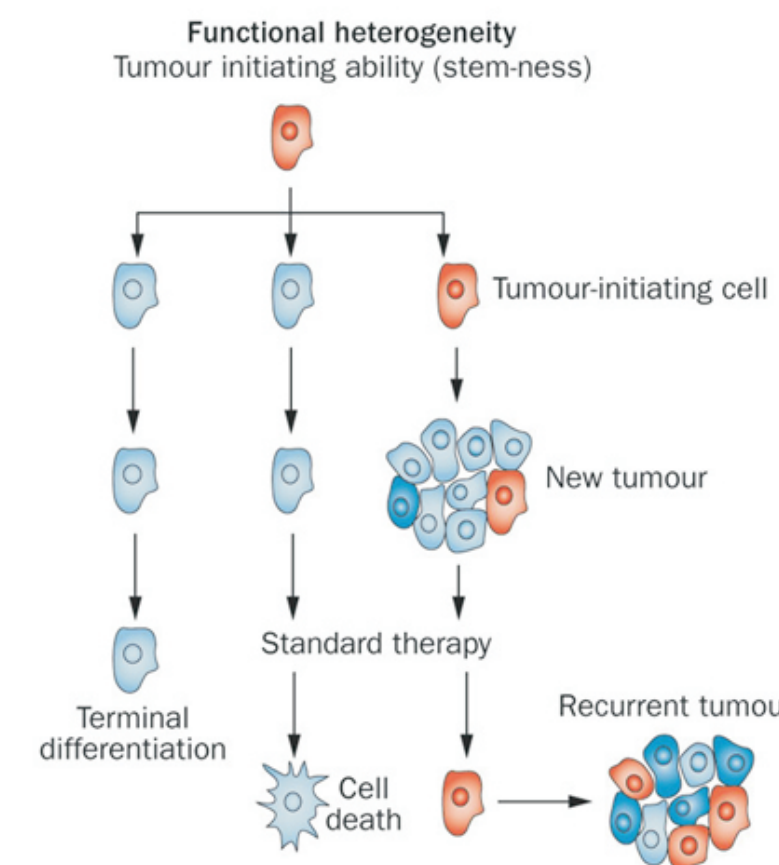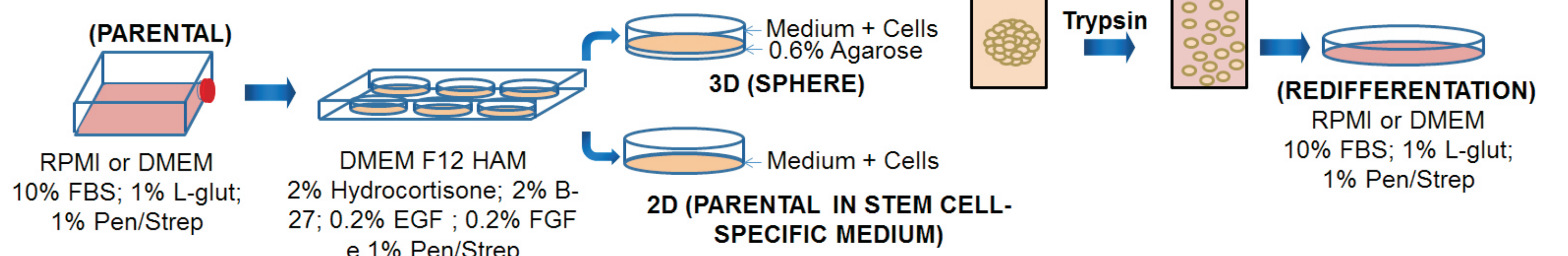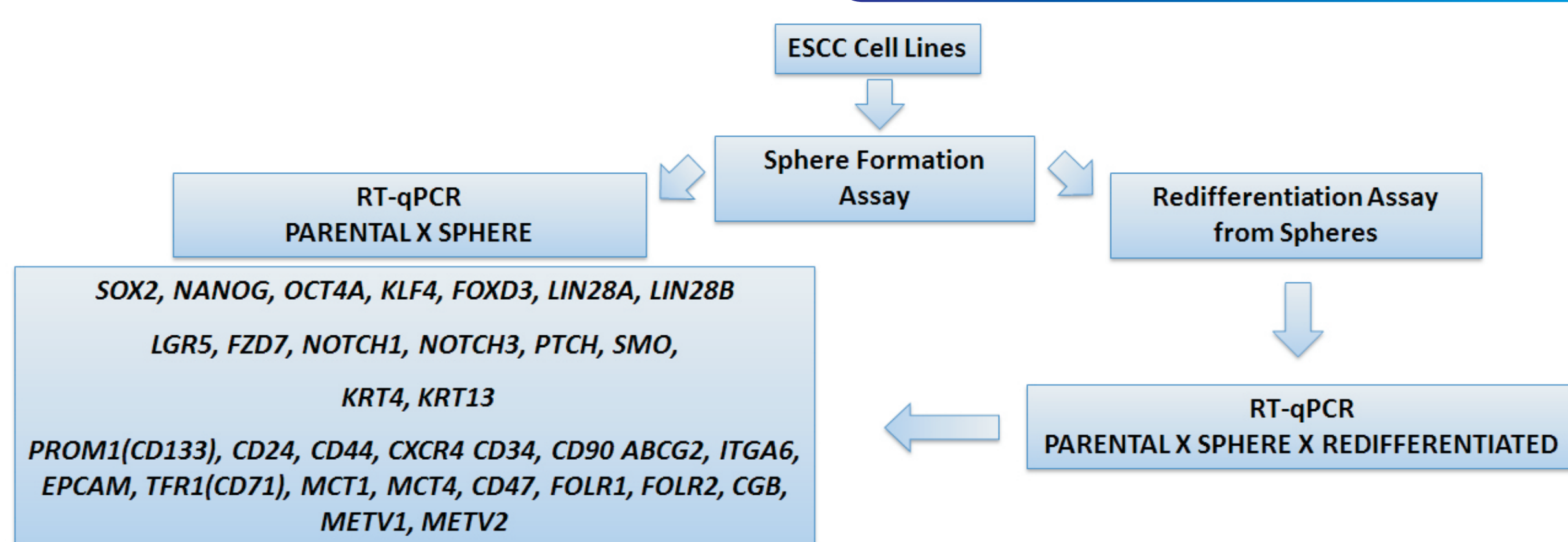

Meric-Bernstam *et al.*, 2012

OBJECTIVE

Isolation and characterization of putative cancer stem cells in esophageal squamous cell carcinoma.

METHODS ANS RESULTS

ESCC Cell Lines

Sphere Formation Assay

RT-qPCR PARENTAL X SPHERE

SOX2, NANOG, OCT4A, KLF4, FOXD3, LIN28A, LIN28B
LGR5, FZD7, NOTCH1, NOTCH3, PTCH, SMO,
KRT4, KRT13
PROM1(CD133), CD24, CD44, CXCR4 CD34, CD90 ABCG2, ITGA6,
EPCAM, TFR1(CD71), MCT1, MCT4, CD47, FOLR1, FOLR2, CGB,
METV1, METV2

Redifferentiation Assay from Spheres

RT-qPCR PARENTAL X SPHERE X REDIFFERENTIATED

(PARENTAL) — RPMI or DMEM 10% FBS; 1% L-glut; 1% Pen/Strep

DMEM F12 HAM 2% Hydrocortisone; 2% B-27; 0.2% EGF ; 0.2% FGF e 1% Pen/Strep

Medium + Cells 0.6% Agarose — 3D (SPHERE)
Medium + Cells — 2D (PARENTAL IN STEM CELL-SPECIFIC MEDIUM)

Trypsin

(REDIFFERENTATION) RPMI or DMEM 10% FBS; 1% L-glut; 1% Pen/Strep

PARENTAL X SPHERE

Common Markers – CXCR4   NANOG   ABCG2   ITGA6

REDIFFERENTATION ASSAY
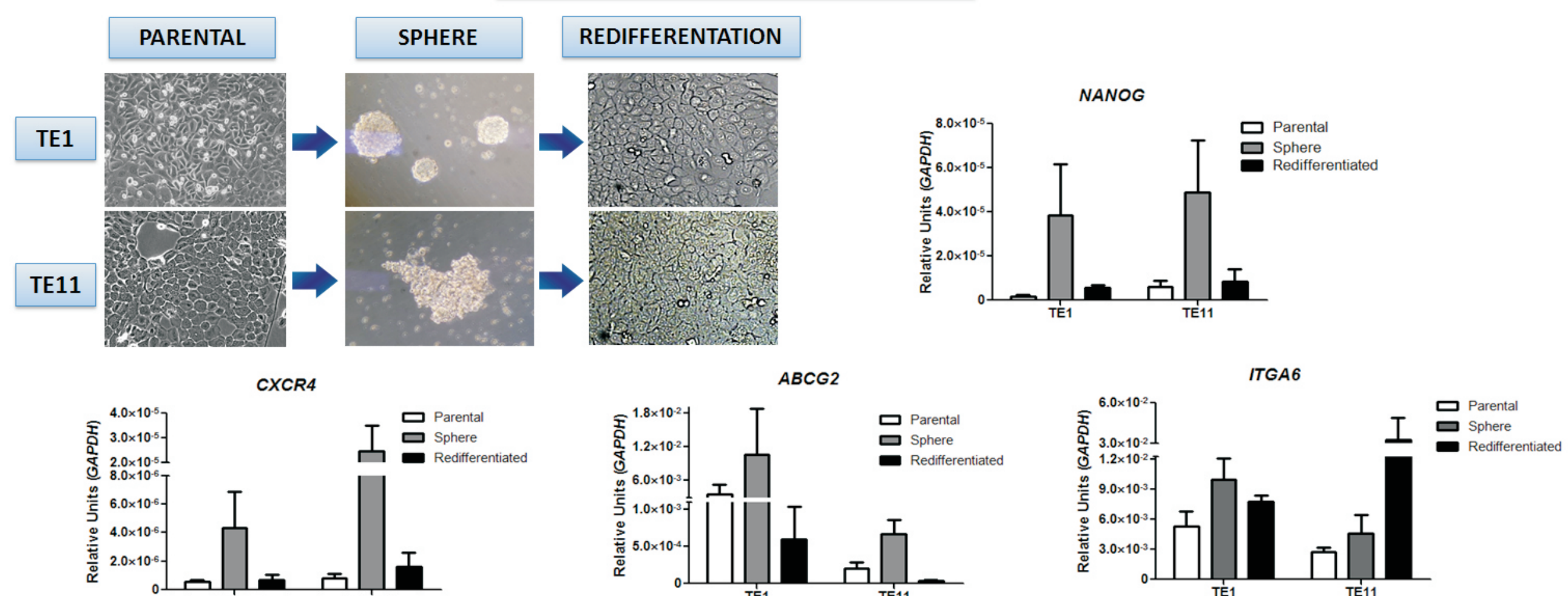

POSSIBLE INFLUENCE OF STEM CELL-SPECIFIC MEDIUM ON GENE EXPRESSION OF POTENCIAL ESCC CANCER STEM CELL MARKERS
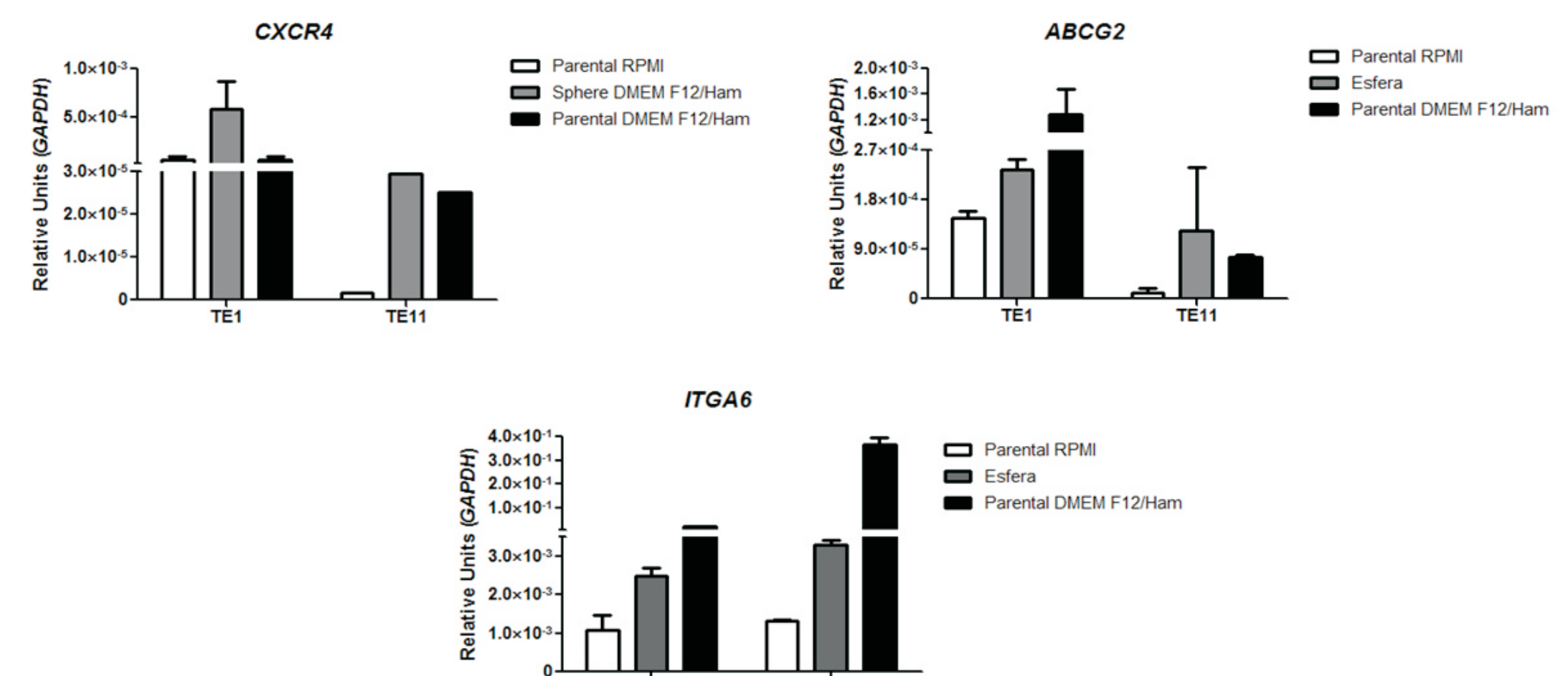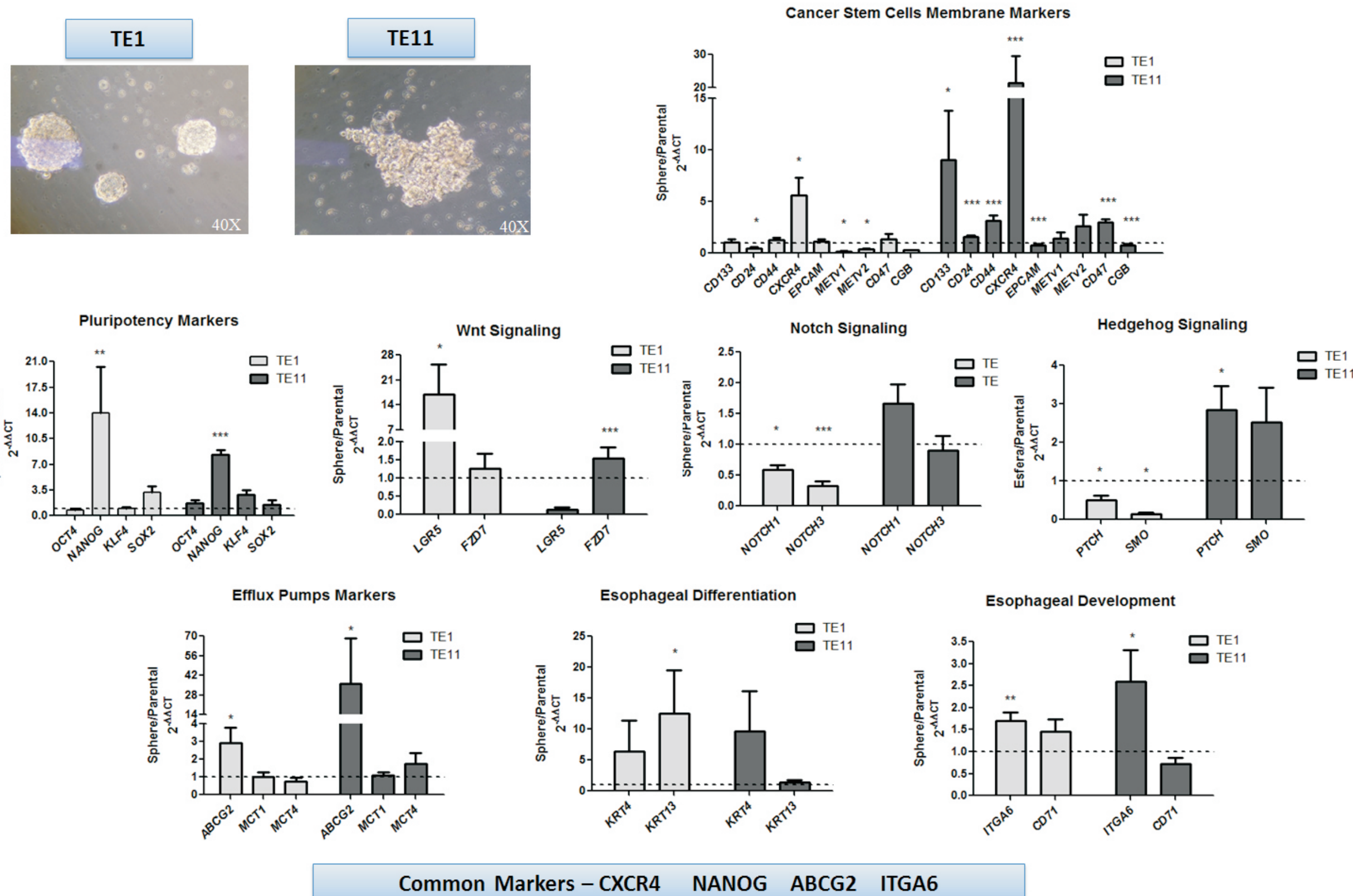
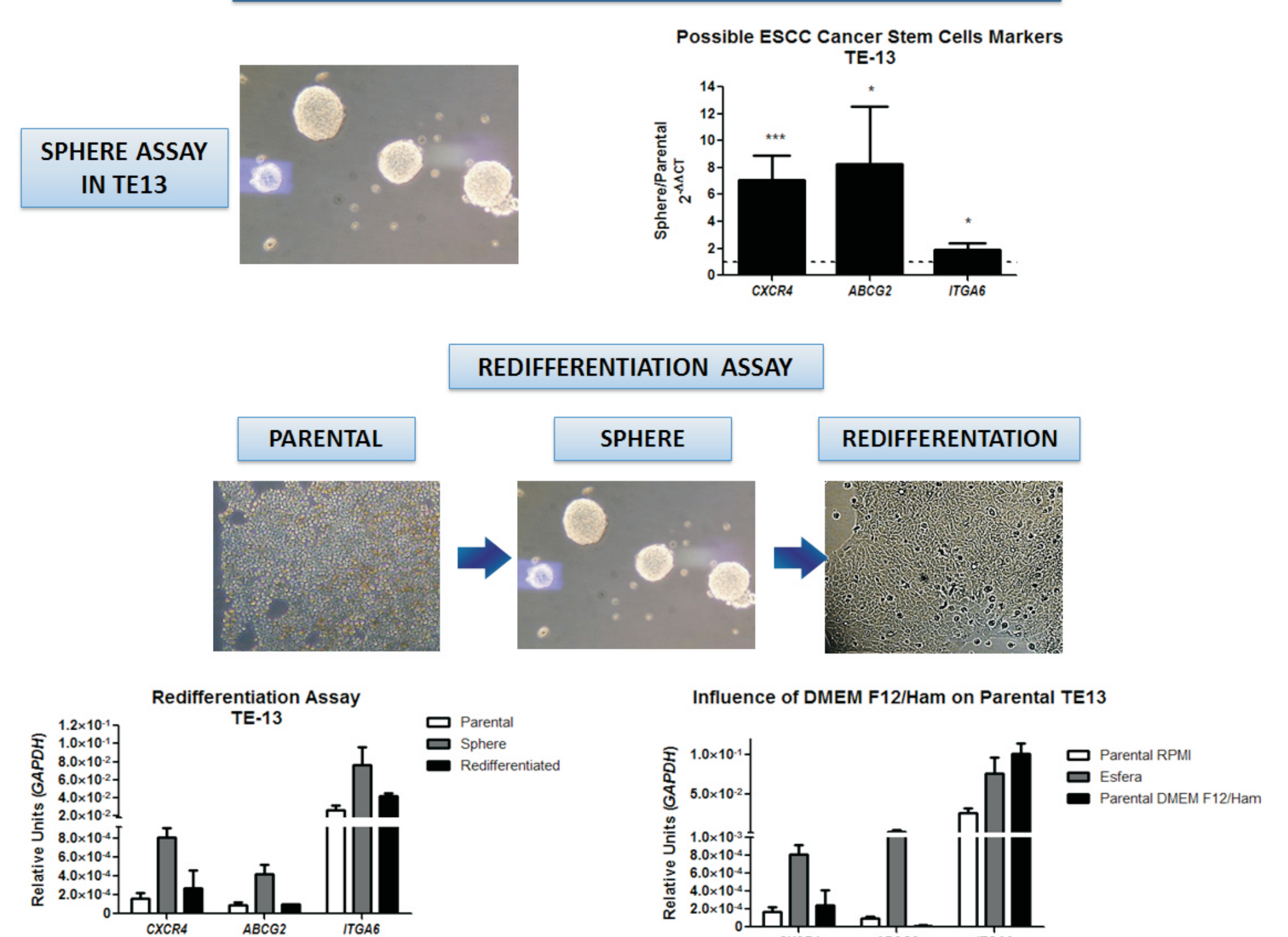### CONFIRMATION IN A THIRD ESCC CELL LINE TE-13

SPHERE ASSAY IN TE13

REDIFFERENTIATION ASSAY

CONCLUSION

- Our data show that CXCR4 and ABCG2 could be possible markers for CSCs in ESCC and must be tested for CSC's population enrichment in xenografic NOD-SCID mouse tumorigenesis assay.

REFERENCES

GLOBOCAN. Cancer incidence and mortality worldwide. (http://wwwdep.iarc.fr) (2012); INCA. Instituto Nacional de Câncer. (http://www.inca.gov.br), 2014; Magee JA, Piskounova E, Morrison SJ. Cancer stem cells: Impact, heterogeneity, and uncertainty. Cancer Cell. 20;21(3):283-96 (2012); Meric-Bernstam F, Mills GB. Overcoming implementation challenges of personalized cancer therapy. Nat Rev Clin Oncol. 9(9) (2012)

PERSPECTIVES

- Validation of protein expression by flow cytometry;
- Cell Sorting, followed by tumorigenic assay in NOD/SCID mice;
- Global molecular characterization of ESCC cancer stem cells by methylome analysis and functional assays.

Ministério da **Saúde**

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