Overexpression of STIM1 and ORAI1 in Circulating Tumor Cells (CTCs) from breast cancer patients

Chrysoula Ntentopoulou[1], Aikaterini Piperidou[1], Anastasia Xagara[2], Athanasios Kotsakis[2], Athina Christopoulou[3], Vassilis Georgoulias[4], Vassilis L Tzounakas[5], Constantinos Stathopoulos[5], and Galatea Kallergi[1]

[1] *Laboratory of Biochemistry and Metastatic Signaling, Division of Genetics, Cell and Developmental Biology, Department of Biology, University of Patras, 26504 Patras, Greece*
[2] *Department of Medical Oncology, University General Hospital of Larissa, 41334 Larissa, Greece*
[3] *Oncology Unit, ST Andrews General Hospital of Patras, GR-26332 Patras, Greece*
[4] *Hellenic Oncology Research Group (HORG), GR-11526 Athens, Greece.*
[5] *Department of Biochemistry, Medical School, University of Patras, 26504 Patras, Greece*

Abstract

Background: Breast cancer is the most frequent malignancy for women worldwide. Metastatic disease is the main cause of patients' death. Therefore, the identification of an effective method for early diagnosis, disease monitoring, and personalized treatment is required. The study of CTCs, major players in metastasis, can contribute to the above goals; however, due to their rarity in the bloodstream, additional biomarkers are required for their detection. The molecules STIM1 and ORAI1, which are implicated in Ca^{2+} signaling through the Store Operated Calcium Entry (SOCE) channel, have been shown to maintain cell migration and metastasis. Thus, they may represent promising biomarkers for the detection of CTCs.

Methods: This study aimed to evaluate the expression of STIM1 and ORAI1 in CTCs isolated from breast cancer patients and to investigate their potential prognostic value. A total of 90 patients were enrolled in this study. 60 samples were isolated with Ficoll density gradient and 30 with the ISET platform, followed by triple immunofluorescence staining using CK (as a CTC marker), STIM1, and ORAI1 antibodies. The samples were analyzed with the VyCAP platform, followed by statistical analysis using the SPSS software.

Results: CTCs were detected in 42% of the Ficoll samples (cytospins). Among these patients, 48% exhibited the (CK+/STIM1+/ORAI1+) phenotype, followed by 39% and 13% with (CK+/STIM1+/ORAI1-) and (CK+/STIM1-/ORAI1-) phenotypes, respectively. Notably, none of the CTCs showcased the (CK+/STIM1-/ORAI1+) phenotype. In ISET samples, 37% of patients harbored CTCs, and 61% of them were positive for (CK+/STIM1+/ORAI1+), while 39% were (CK+/STIM1+/ORAI1-). Cytospins analysis revealed that patients with a detected response to treatment had significantly longer progression-free survival (PFS: $p<0.001$) compared to non-responders. ISET-based analysis showed that patients with the (CK+/STIM1+/ORAI1+) phenotype had longer PFS ($p=0.014$).

Conclusions: Overall, STIM1 and ORAI are overexpressed in CTCs derived from breast cancer patients. Surprisingly, their expression was positively correlated with patients' outcomes. The ISET platform demonstrates enhanced prognostic relevance regarding the detection and characterization of CTCs. Nevertheless, further comprehensive studies are required to confirm the clinical significance of these biomarkers.

Introduction

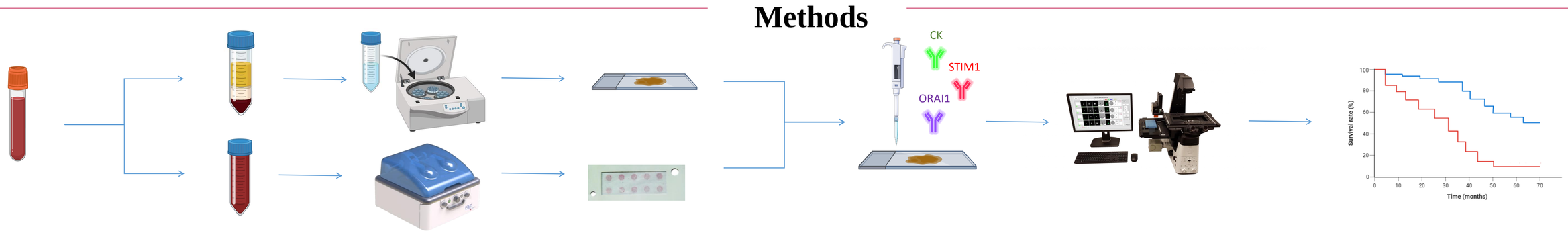
Breast cancer is a heterogeneous disease with important participation of both genetic and environmental factors. Their interaction controls a complex pathogenesis, leading to difficulties in determining the appropriate therapeutic approach.[1]. Approximately 90% of deaths are linked to tumor metastasis, while it is estimated that a quarter of patients will develop distant metastasis[2].

In recent years, liquid biopsy has made significant progress, enabling real-time disease monitoring. The use of CTCs as a liquid biopsy tool draws unique information to the changes and progression of the tumor. The presence of CTCs in the peripheral blood has a crucial role in metastasis[3]. The molecular heterogeneity of CTCs necessitates the phenotypic characterisation of these cells, aiming at the distinction of subpopulations with high metastatic dynamic and the evaluation of new prognostic biomarkers[4].

Intracellular Ca^{2+} homeostasis is regulated by the SOCE ion channel, which is the main Ca^{2+} entry in non-excitable cells such as cancer cells and is comprised of the molecules STIM1 located in the ER membrane and ORAI1 embedded in the plasma membrane. Specifically, STIM1 acts as a Ca^{2+} sensor, detecting changes in the intracellular environment. The decrease in Ca^{2+} in intracellular stores leads to the binding of STIM1 to ORAI1 molecules and the opening of the SOCE channel for Ca^{2+} entry[5]. When SOCE is activated in breast cancer, increased expression of STIM1 and ORAI1 is observed.The overactivation of SOCE has been implicated in tumorigenesis and disease progression by promoting cell proliferation, resistance to apoptosis, cell migration and metastasis[6].

In this study, we investigated the expression of STIM1 and ORAI1 in CTCs from breast cancer patients and correlated the results with the patients' clinical data in order to identify the potential predictive value of these molecules.

Methods



Results

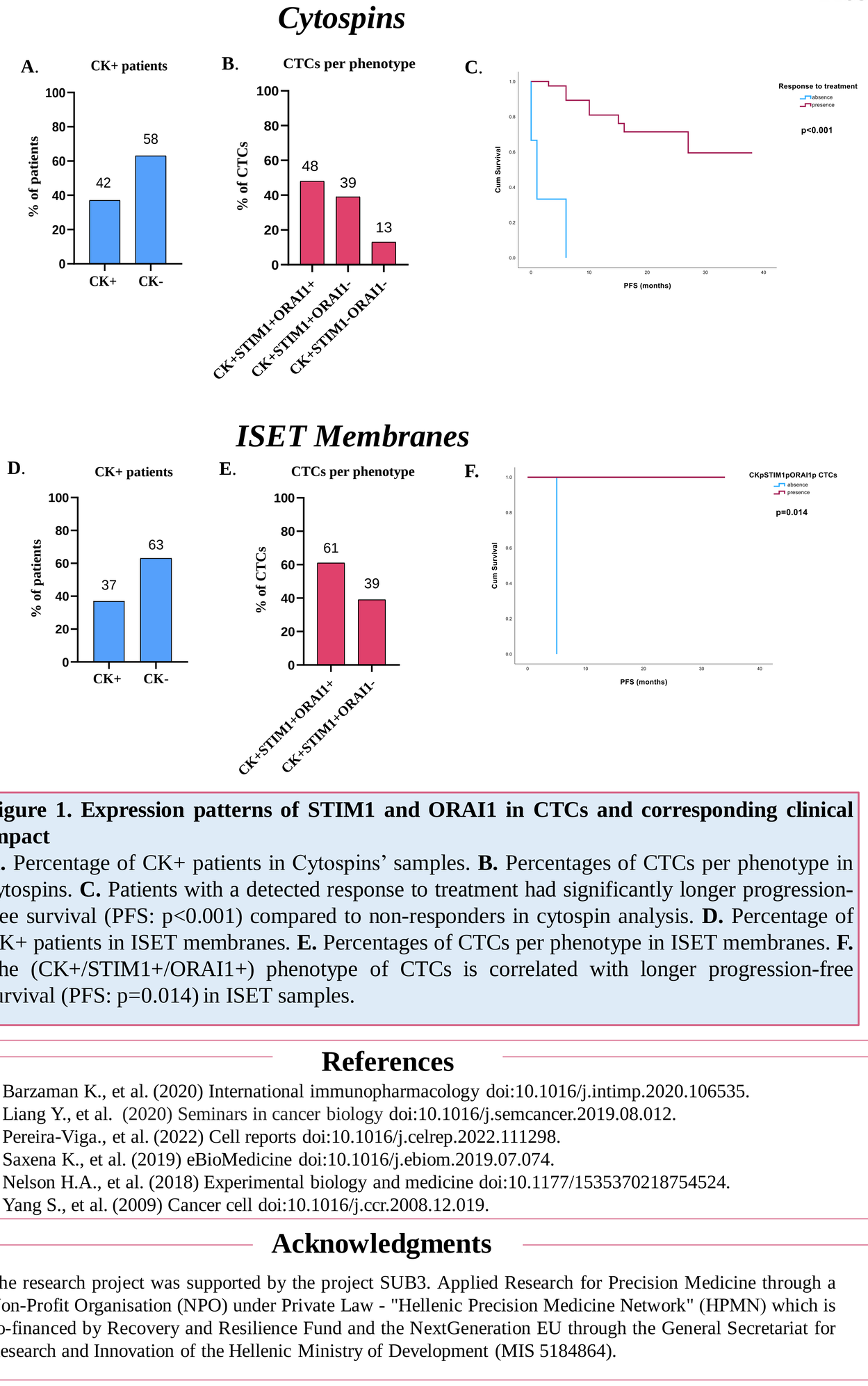


Figure 1. Expression patterns of STIM1 and ORAI1 in CTCs and corresponding clinical impact
A. Percentage of CK+ patients in Cytospins' samples. **B.** Percentages of CTCs per phenotype in cytospins. **C.** Patients with a detected response to treatment had significantly longer progression-free survival (PFS: $p<0.001$) compared to non-responders in cytospin analysis. **D.** Percentage of CK+ patients in ISET membranes. **E.** Percentages of CTCs per phenotype in ISET membranes. **F.** The (CK+/STIM1+/ORAI1+) phenotype of CTCs is correlated with longer progression-free survival (PFS: $p=0.014$) in ISET samples.

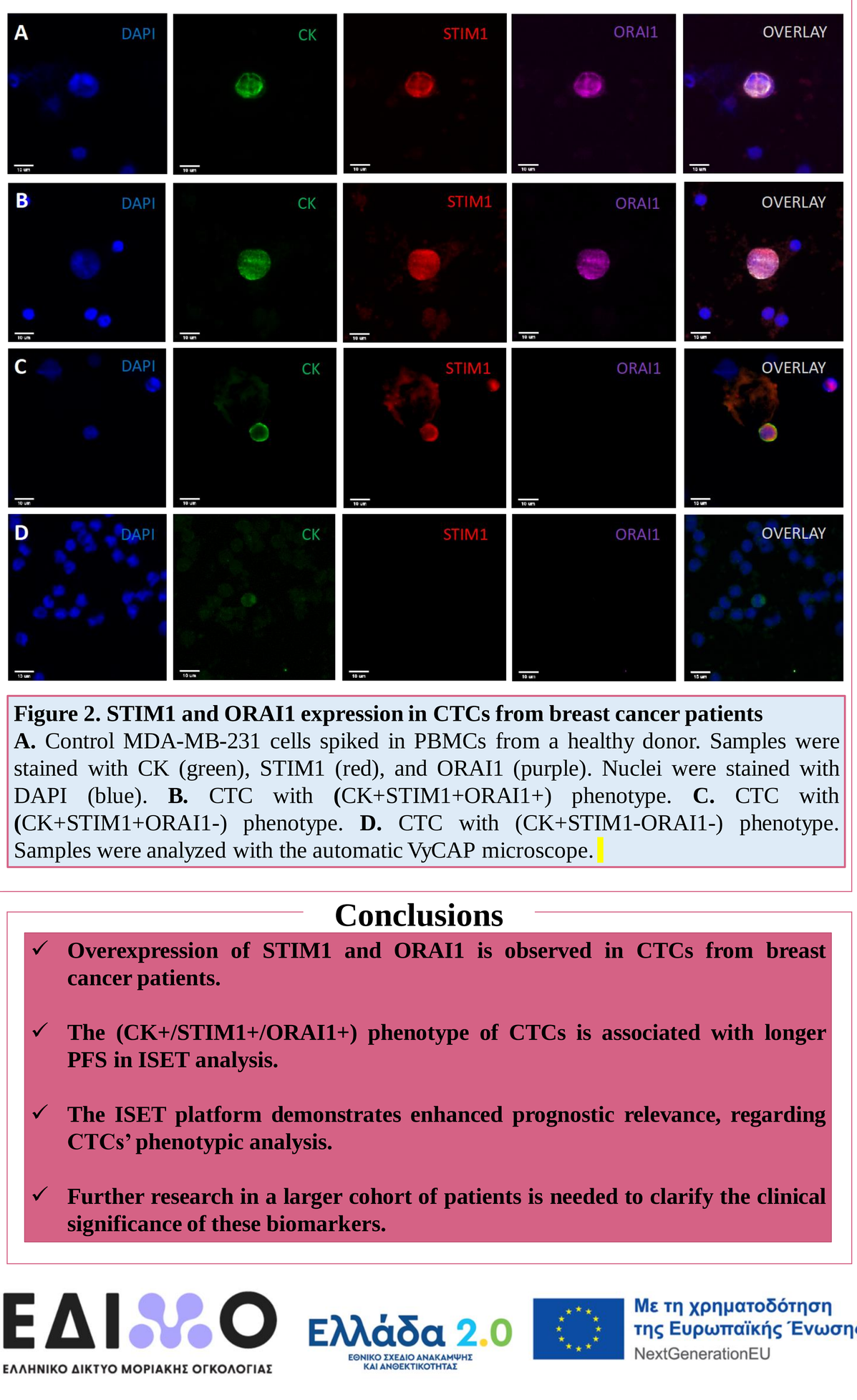


Figure 2. STIM1 and ORAI1 expression in CTCs from breast cancer patients
A. Control MDA-MB-231 cells spiked in PBMCs from a healthy donor. Samples were stained with CK (green), STIM1 (red), and ORAI1 (purple). Nuclei were stained with DAPI (blue). **B.** CTC with **(**CK+STIM1+ORAI1+) phenotype. **C.** CTC with **(**CK+STIM1+ORAI1-) phenotype. **D.** CTC with (CK+STIM1-ORAI1-) phenotype. Samples were analyzed with the automatic VyCAP microscope.

Conclusions

- ✓ **Overexpression of STIM1 and ORAI1 is observed in CTCs from breast cancer patients.**
- ✓ **The (CK+/STIM1+/ORAI1+) phenotype of CTCs is associated with longer PFS in ISET analysis.**
- ✓ **The ISET platform demonstrates enhanced prognostic relevance, regarding CTCs' phenotypic analysis.**
- ✓ **Further research in a larger cohort of patients is needed to clarify the clinical significance of these biomarkers.**

References

1. Barzaman K., et al. (2020) International immunopharmacology doi:10.1016/j.intimp.2020.106535.
2. Liang Y., et al. (2020) Seminars in cancer biology doi:10.1016/j.semcancer.2019.08.012.
3. Pereira-Viga., et al. (2022) Cell reports doi:10.1016/j.celrep.2022.111298.
4. Saxena K., et al. (2019) eBioMedicine doi:10.1016/j.ebiom.2019.07.074.
5. Nelson H.A., et al. (2018) Experimental biology and medicine doi:10.1177/1535370218754524.
6. Yang S., et al. (2009) Cancer cell doi:10.1016/j.ccr.2008.12.019.

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