Evaluation of the oncogenic potential of the *ESR1-Y537C* variant as a prospect for melanoma induction and development

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Introduction

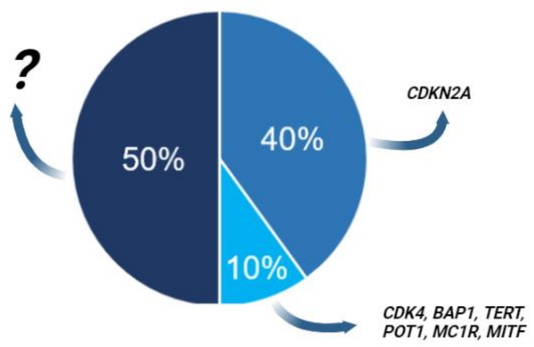
- Melanoma is a malignant neoplasm of melanocytes and is the third most common type of skin cancer after basal and squamous cell carcinomas[1]. Although melanoma comprises the minority of the cases of all cutaneous malignancies (5%), it is the most aggressive skin cancer not only due to its high metastasis potential, but also because it accounts for most skin cancer deaths [2].

- The most significant risk factor for developing melanoma are somatic mutations due to exposure to ultraviolet light; nonetheless, it is estimated that approximately 10% of melanoma cases are of familial origin.[3].

- Germline variants such as those in *CDKN2A, CDK4*, *BAP1*, *TERT*, *POT1*, *MC1R*, and *MITF* explain about 50% of familial cases; however, the genetic cause of the remaining percentage is unknown[4].

- It has been suggested that the occurrence of progressive melanoma may be influenced by the expression of estrogen receptors[5].

- In recent years, our group in collaboration with the GenoMEL consortium found 13 novel candidate genetic variants from whole-genome sequencing data of 314 patients from 135 melanoma-prone families. Of these, the estrogen receptor 1 (*ESR1*) Y537C variant stood out as it had previously been reported as a somatically acquired driver in breast cancer.



Objective

Due to the presence of the *ESR1-Y537C* variant in one melanoma-prone family, and the fact that the oncogenic potential of this mutation has not been proven in this type of cancer, the aim of this work is to assess the oncogenic potential of the mutation in NIH3T3 cells to provide enough evidence for further studies in melanocytic and melanoma cells.

Methods

- NIH3T3 cells transfection was performed using the Lipofectamine 2000 kit and different vectors derived from the pBABE plasmid (fig. 1). Five groups were generated after the transfection process:

  pBabe (-) = Wild type (negative control)

  pBabe (+) backbone = Cassette (negative control)

  pBabe (+), *K-RAS-G12V* gene (+) = K-RAS (positive control)

  pBabe (+), *ESR1* gene (+) = ESR1

  pBabe (+), *ESR1-Y537C* gene (+) = ESR1-Y537C

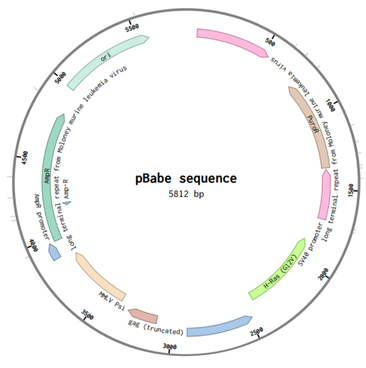


Figure 1. Plasmid pBabe sequence. Taken from Morgenstern, et al. 1990.

- After transfection, each group was subjected to a series of procedures (fig. 2). A) A cell proliferation assay was conducted for 3 different times: 24hrs, 48hrs, and 72 hrs. B) The focus formation assay was carried out after a period of 12 days. C) For the scratch wounding assay, all groups were analyzed over a period of 6 hours. The whole experiment was performed twice.

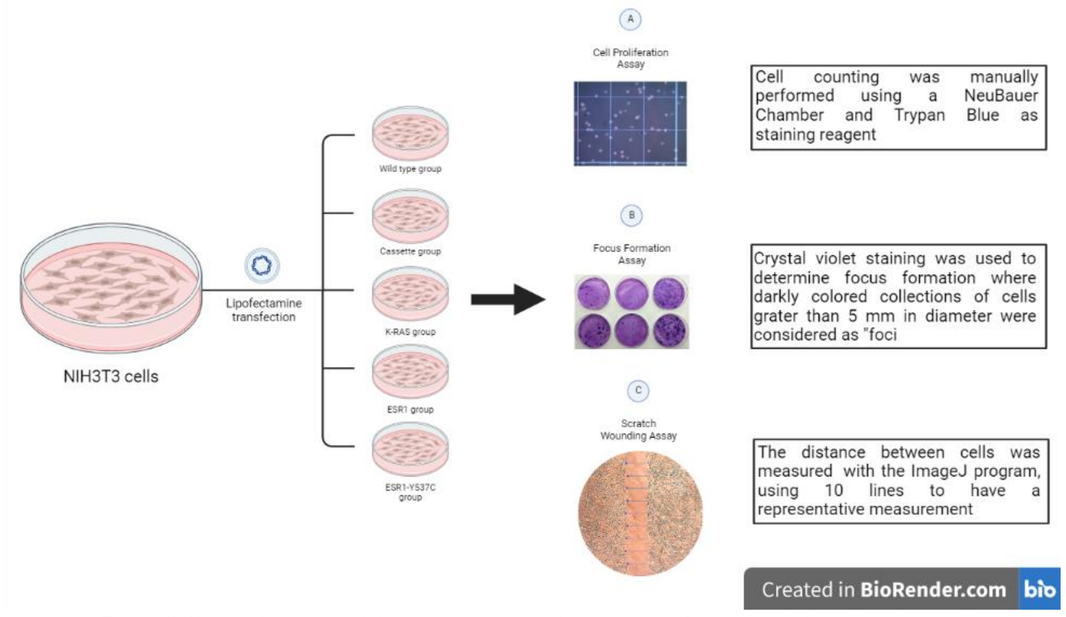


Figure 2. Schematic representation of the experimental procedure. A brief description of each assay is provided.

- Data were analyzed by set using the R-Studio version 4.2.2 software. Linear regression models were performed to assess whether conditions, such as group and time, were related to cell proliferation. A two-way ANOVA was also performed to analyze variances between conditions. The significance level was considered to be $P < 0.05$.

Results

Cell proliferation assay

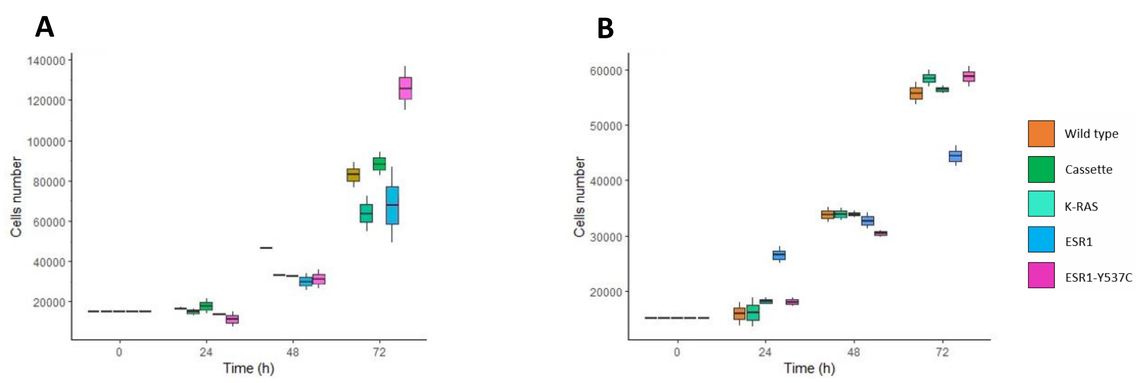


Figure 3. Cell proliferation was conducted 3 different times: 24hrs, 48hrs, and 72 hrs. A) Set 1. B) Set 2. No significant difference was found for any group in both sets.

Focus formation assay

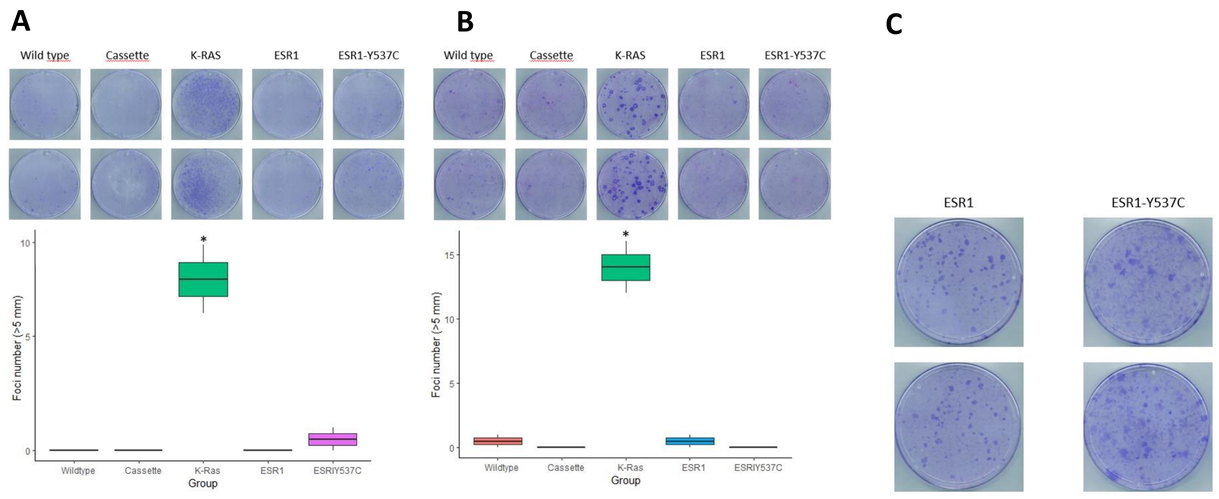


Figure 4. The focus formation assay was carried out after a period of 12 days: A) Set 1. B) Set 2. *: K-RAS proved to be the significantly different group in both sets ($P < 0.05$). C) Foci formation induced by *ESR1* and *ESR1*-Y537C at 18 days.

Scratch wounding assay

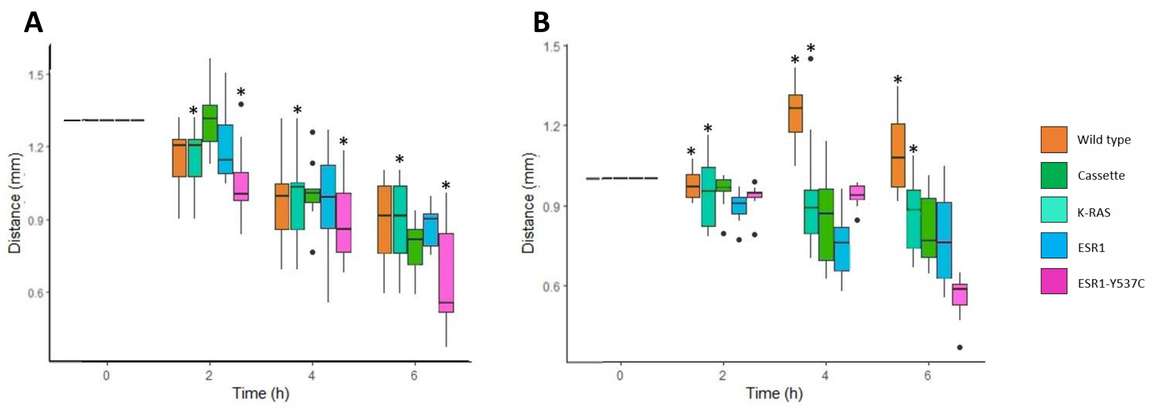


Figure 5. Scratch wounding assay was performed in 4 periods: 0 hrs, 2 hrs, 4hrs, and 6 hrs after the initial scratch. A) Set 1. *: Cassette and ESR1-Y537C groups proved to be significantly different ($P < 0.05$). B) Set 2. *: Wild type and cassette group proved to be significantly different ($P < 0.05$). Outliers are presented as black circles.

Conclusion

Evidence from these experiments suggest so far that the *ESR1-Y537C* variant has an oncogenic effect on NIH3T3 cells, due to the faster time to healing and the foci formation at 18 days; however, a third replicate is underway in order to increase the statistical power. Likewise, evaluation in melanoma and melanocytic cells is required to elucidate its role in the induction and development of melanoma.

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References

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