IMPLICATION OF CEMIP IN THE MALIGNANT PROGRESSION OF CUTANEOUS MELANOMA

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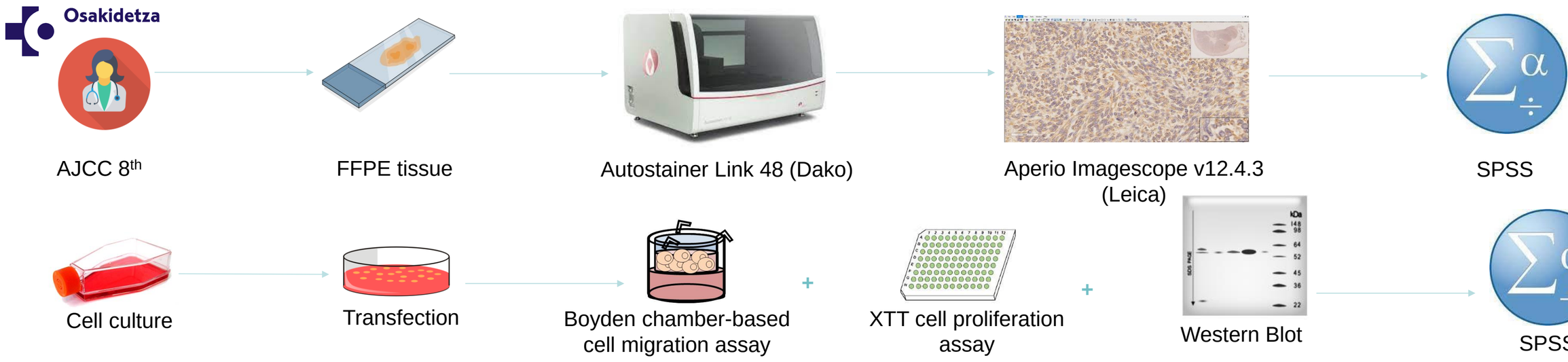
INTRODUCTION

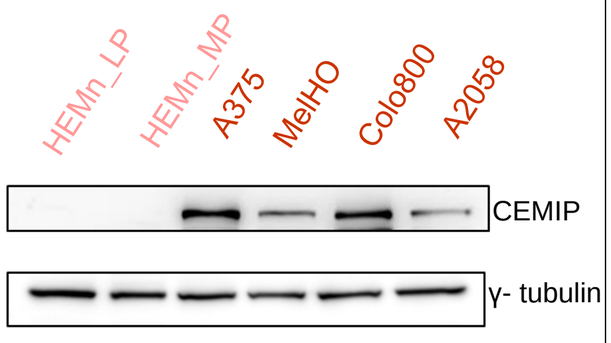
Malignant cutaneous melanoma is the deadliest form of skin cancer due to its high rate of metastasis, even when identified in early stages (Bajaj et al., 2020). The lack of effective strategies for metastatic melanoma supports the need for a more profound understanding of metastasis-driving molecules and their role as accurate prognostic biomarkers. Based on a previous proteomic study, we have identified CEMIP (*cell migration inducing protein, hyaluronan binding*) as a protein of interest. This protein has been shown to modulate cellular proliferation, motility as well as to alter Epithelial-Mesenchymal Transition (EMT)-related proteins (Fink et al., 2015); moreover, it has been associated with the poor survival rate of patients suffering from several types of cancer (Rodrigues et al., 2019) although little is known regarding melanoma. The aim of this study is to investigate the role of CEMIP on the malignant progression of cutaneous melanoma.

MATERIAL AND METHODS



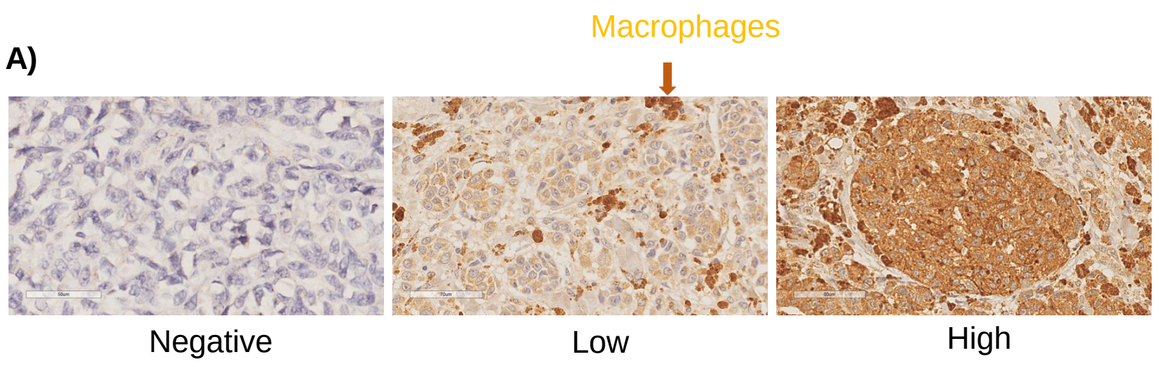
RESULTS

CEMIP is enriched in melanoma cell lines



Cellular CEMIP extracted from melanocytes and melanoma cell lines was evaluated by Western Blot. CEMIP protein level was enriched in melanoma cells lines compared to melanocytes.

High cellular CEMIP level is more frequently observed on lesions of patients that developed metastasis



B)

		METASTASIS		
		No	Yes	Total
STAINING	Negative	8	7	15
	Low	17	17	34
	High	7	24	31
Total		32	48	80

Stage I-IV melanoma samples

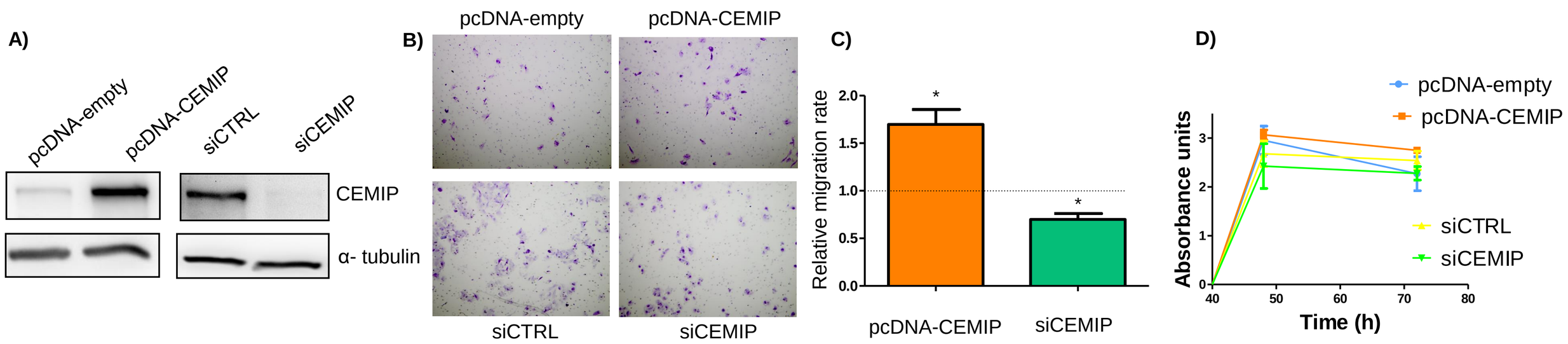
C)

		METASTASIS		
		No	Yes	Total
STAINING	Negative	8	6	14
	Low	17	5	22
	High	7	13	20
Total		32	24	56

Early-stage (I-II) melanoma samples

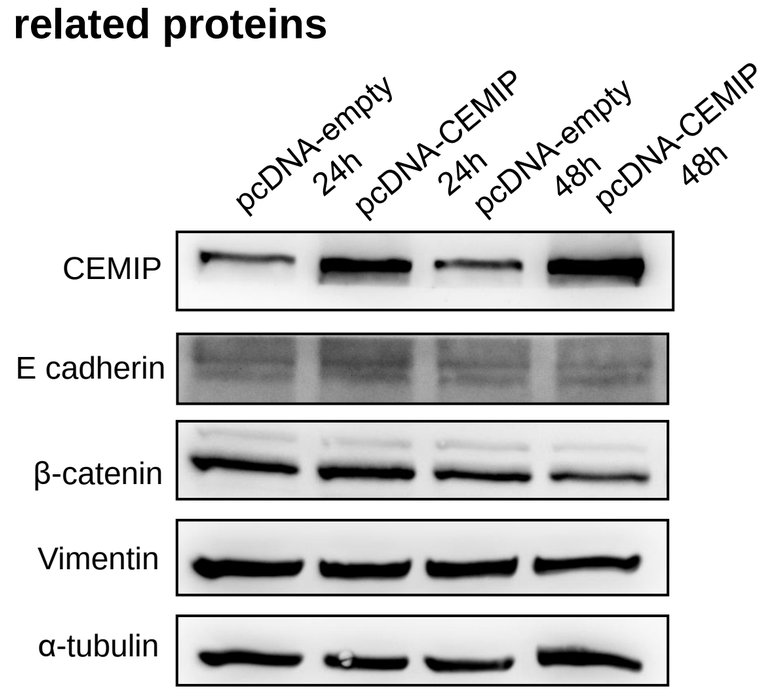
128 FFPE tissue samples including benign (nevi) and malignant (melanoma, stages I-IV) lesions were analysed by immunohistochemistry (IHC). **A)** Examples of **staining intensities** for sample classification. Samples were analysed by 3 independent observers. **B)** Contingency table showing **staining distribution of stage I-IV samples** classified according to their capacity to develop metastasis. Both variables exhibited a positive correlation of 0,284 according to Cramer's V (if considered as a nominal variable; Chi-square p=0,04). **C)** Contingency table showing **staining distribution of early stage (I-II) melanoma samples** classified according to their progression (i.e. those that developed metastasis during the follow-up vs those that did not). Both variables exhibited a positive correlation of 0,369 according to Cramer´s V with a significance of p=0,022 according to the Chi-square analysis. No significant difference was observed when analysed by sample staging or including benign lesions.

Cellular CEMIP level modulates migration capacity but not proliferation of human melanoma cells



Colo800 cells were transfected with overexpression plasmid or siRNA in order to modify cellular CEMIP level. Migration was determined at 28h (48h post transfection) while proliferation was analysed at 48h and 72h post-transfection. **A)** CEMIP overexpression and silencing were confirmed by Western Blot. **B)** Representative images of processed transwell membranes containing **cells stained** with crystal violet. **C)** Column graph shows **enhanced and impaired migration capacity** upon CEMIP **overexpression** and **silencing**, respectively (dash line represents control migration rate. Mean and SD of three independent experiment performed in triplicates. Student-t p=0.01). **D) No significant differences** were observed **on proliferation** between the control and cells with altered levels of CEMIP (mean and SD of three independent experiments performed in triplicates. Student-t p>0.05).

CEMIP overexpression has no effect over EMT-related proteins



Whole cell lysates were analyzed by Western Blot upon cell transfection with overexpression plasmid (24h and 48h post transfection) in order to evaluate EMT-related protein markers. E cadherin, β-catenin and vimentin showed no significant differences between the control and cells with altered levels of CEMIP.

CONCLUSIONS

Cellular CEMIP staining could be considered a prognostic biomarker for early stage melanomas with metastatic risk.

Current data support the implication of CEMIP in the metastatic progression of melanoma by promoting cellular migration through molecular pathways distinct to classic EMT.

References

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