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INTRODUCTION AND OBJECTIVE

- Thyroid cancer (TC) is the most prevalent endocrine malignancy, and its incidence and mortality rates have been increasing in the last three decades all over the world.
- TC frequently present aberrant expression of different gene products, which could be related to tumor progression. Our previous reports have shown that osteopontin-a (OPNa) splice variant is overexpressed and associated with TC progression features.
- In this model, the signaling pathways by which OPN function are currently unknown.
- This study aimed to investigate the role of OPNa on activating signaling pathways by which this variant induces tumor progression.

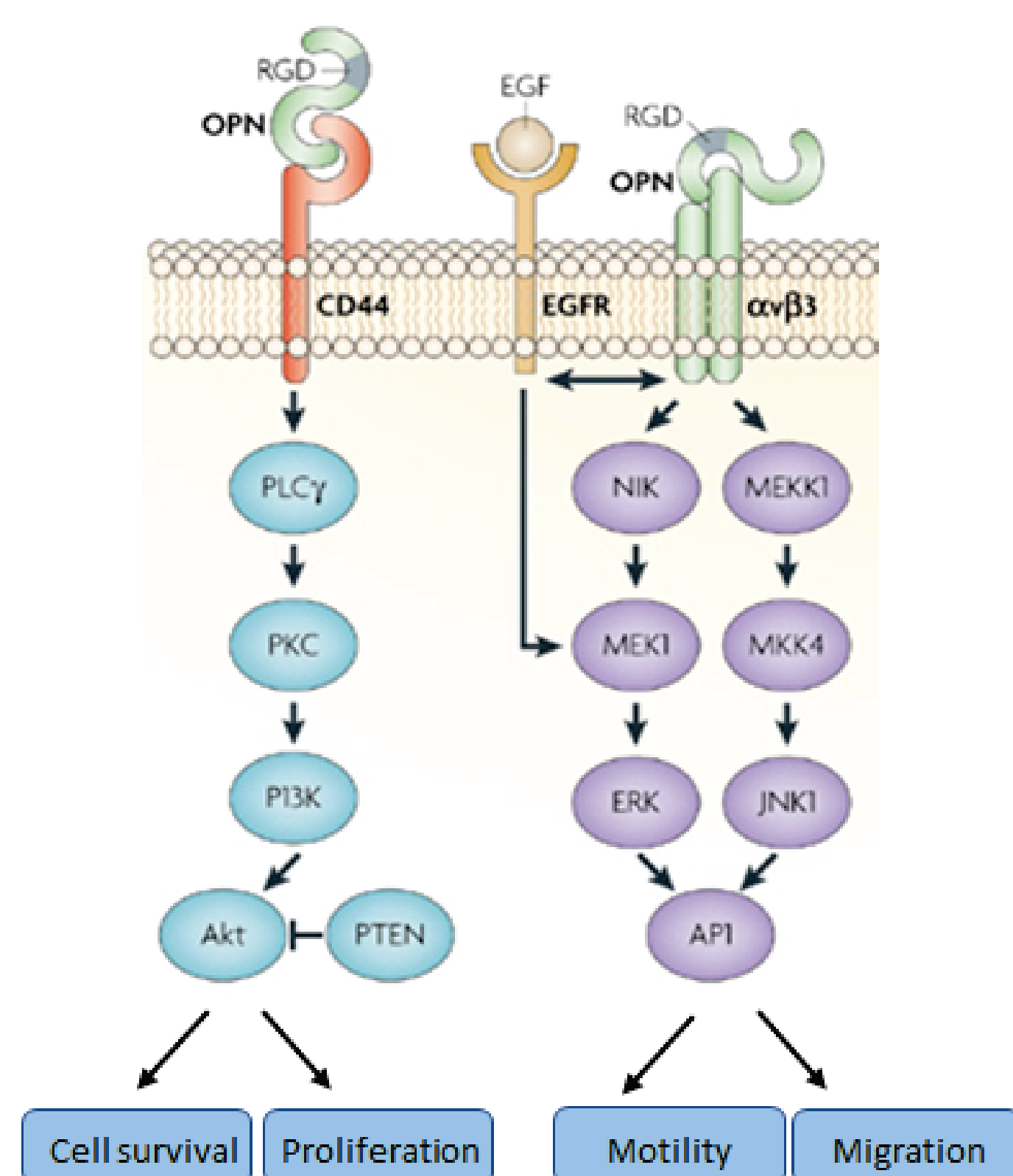
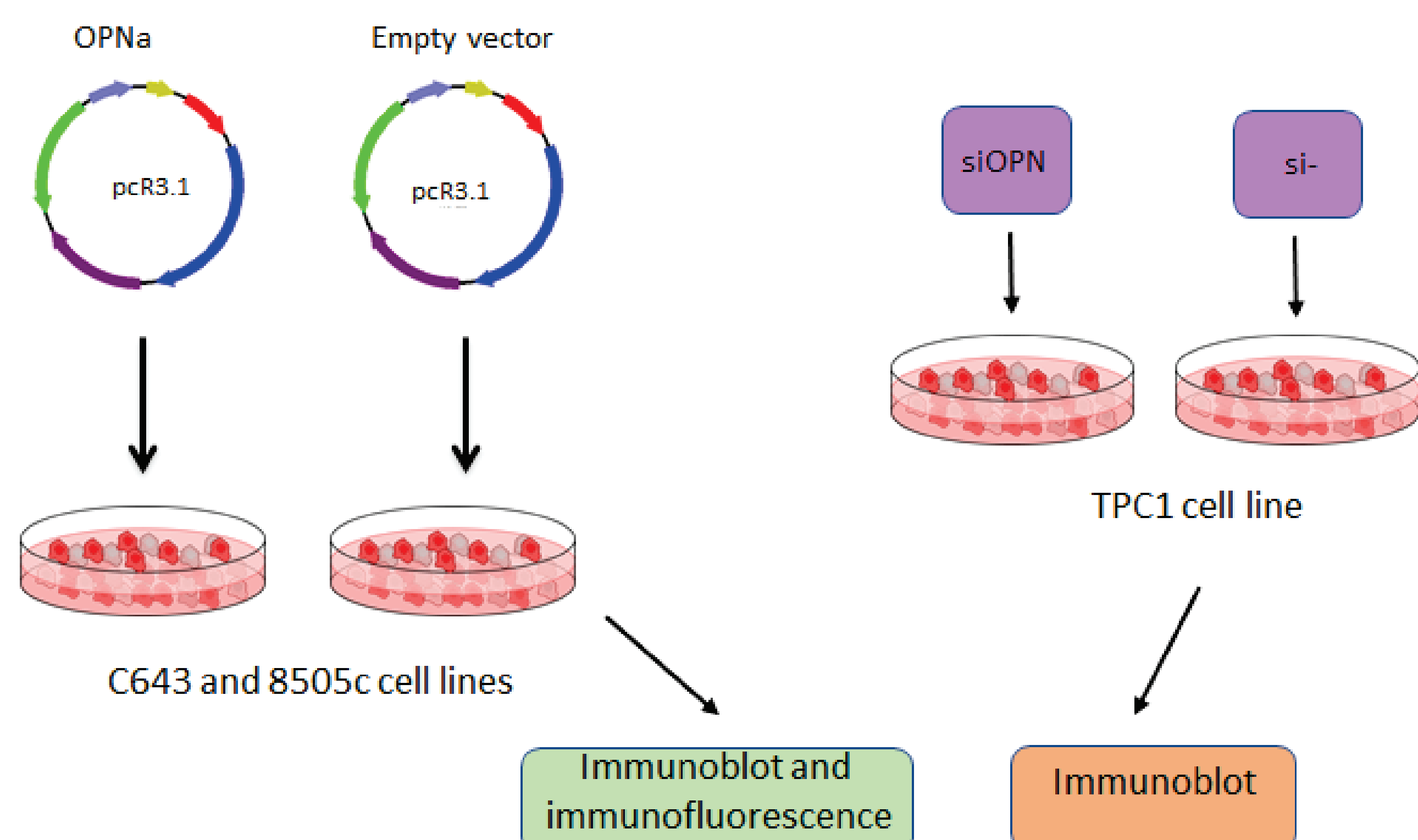


Figure 1: Signalling pathways activated upon OPN binding to CD44 and integrin receptors. OPN can interact with several integrins and can also interact with the CD44 family of receptors. Some of these complexes are able to mediate cell survival through (PKC–PI3K–Akt) pathway activation that leads to anti-apoptotic signals in tumor cells. OPN-induced Akt phosphorylation can be blocked by the tumor suppressor PTEN. Moreover, motility through the activation of the canonical $\alpha v\beta 3$ integrin pathway where both nuclear factor-inducing kinase ERK and MEK1-JNK1 signalling promote cell migration by activating AP1-dependent gene expression. Upon binding to $\alpha v\beta 3$, OPN also stimulates EGFR transactivation, ERK phosphorylation and AP1 activation. Adapted from: Bellahcène et al., 2008 (Nature Reviews Cancer).

MATERIAL AND METHOD

- The protein levels of PI3K/AKT and MAPK pathways (PKC, pPKC, ERK, pERK, AKT and pAKT) were analyzed by immunoblot in TC cell lines overexpressing OPNa (c643-OPNa and 8505c-OPNa).
- TPC1 cell line was used to knockdown OPN and analyze levels of some proteins involved in these signaling pathways.
- The levels of $\alpha v\beta 3$ and CD44, which are OPN cell surface receptors, were verified by immunohistochemistry in c643-OPNa cell line.



RESULTS

Expression $\alpha v\beta 3$ integrin was identified on the plasma membrane of c643-OPNa cells, as well as in the cytoplasm and nucleus compartments. Moreover, we also found that OPNa overexpression induced PKC phosphorylation in both c643 and 8505c TC cell lines.

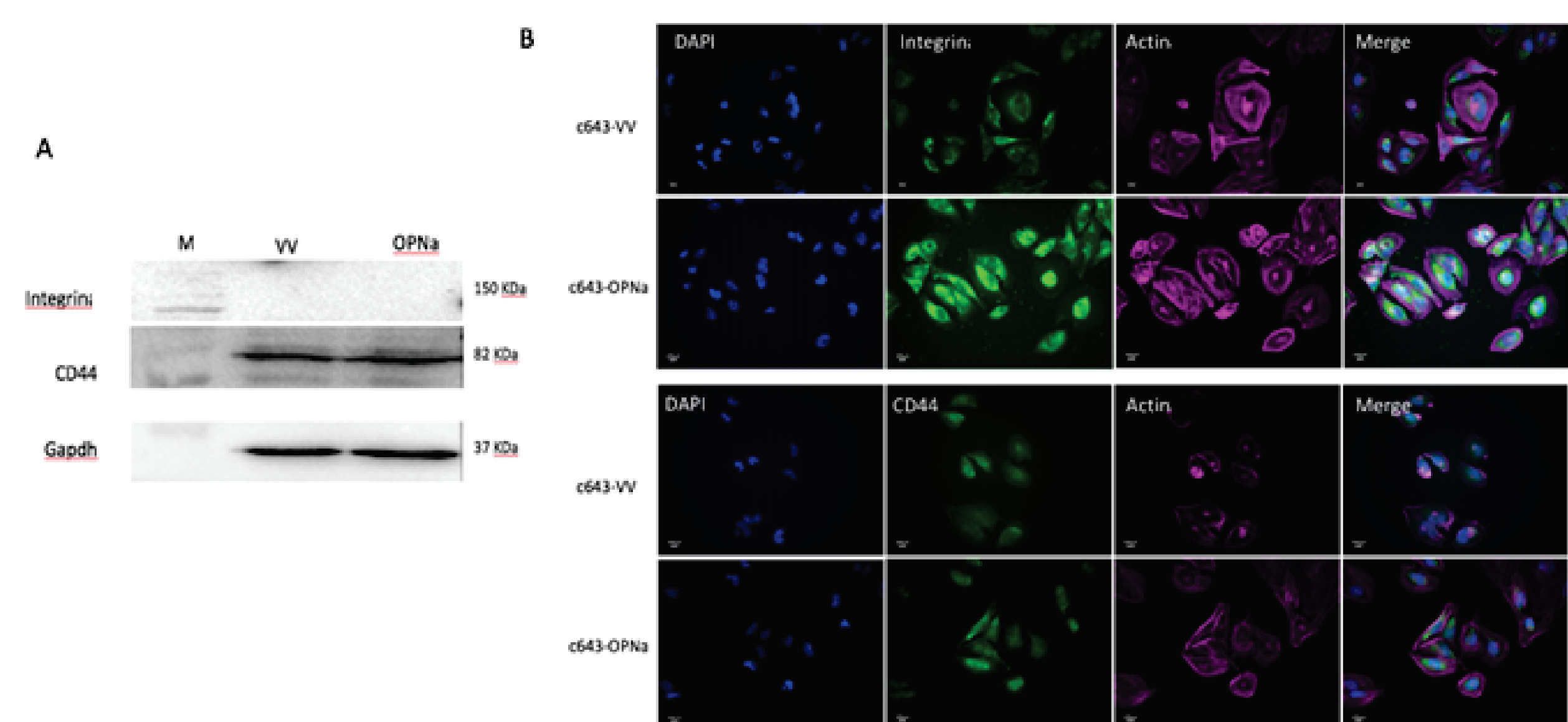


Figure 2: Evaluation of integrin ($\alpha v\beta 3$) and CD44 receptors in c643-OPNa and c643-VV cells. The levels of integrin ($\alpha v\beta 3$) and CD44 receptors in c643-OPNa and c643-VV cells was analyzed by immunoblot and immunofluorescence. A) Representative image of the $\alpha v\beta 3$ and CD44 protein levels from c643-VV and c643-OPNa cells. Representative GAPDH expression pattern is shown. B) Immunofluorescence assay of c643-OPNa and c643-VV cells stained with DAPI (blue), integrin (green) and CD44 (violet). In all samples, colocalization of DAPI, integrin and actin staining were observed.

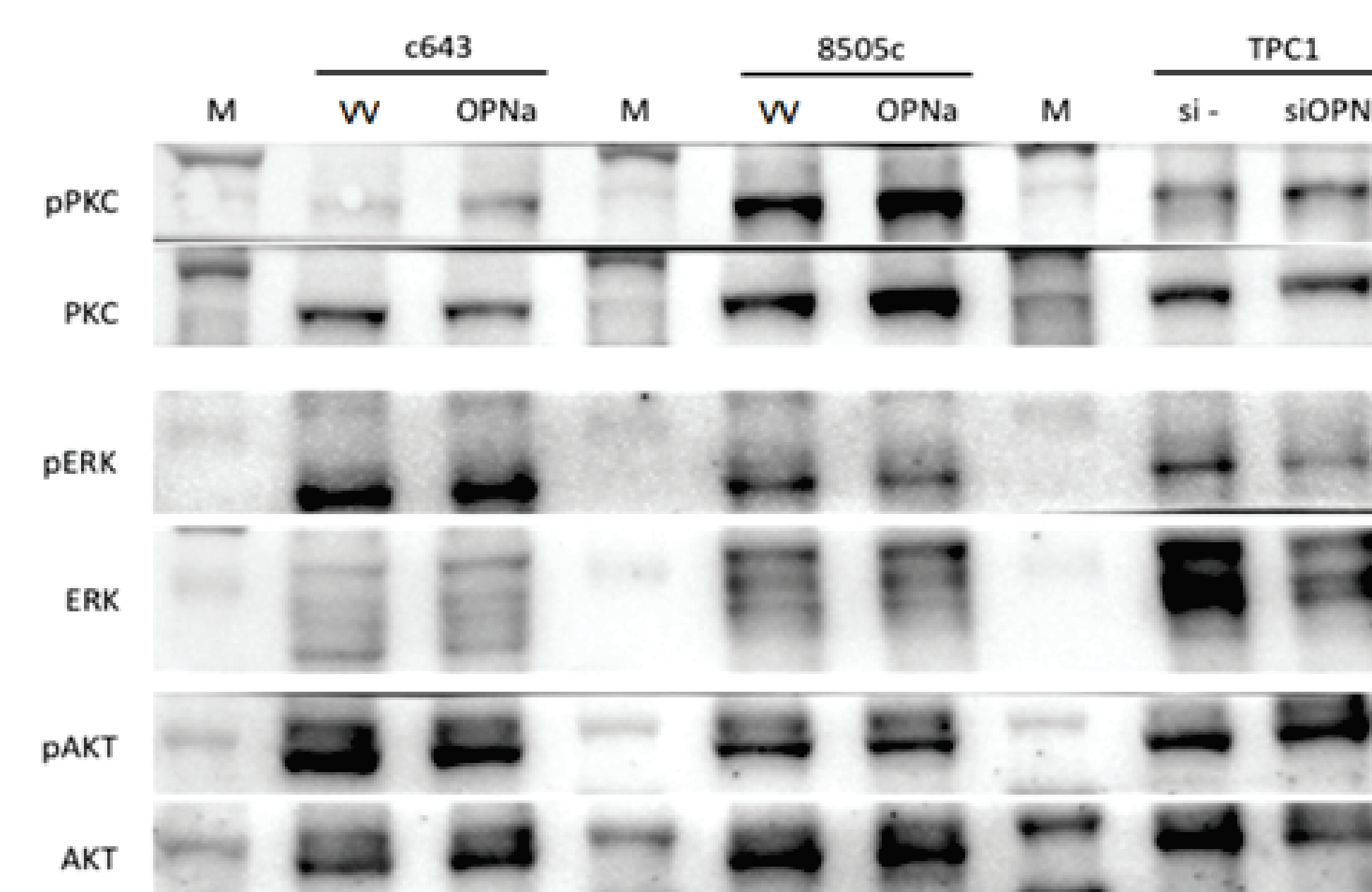


Figure 3: Immunoblot analysis of pPKC, pERK and pAKT activation in response to OPNa overexpression (c643 and 8505c cells) and OPN silencing (TPC1 cells). Representation of the phosphorylated protein levels (pPKC, pERK and pAKT) and their respective total proteins (PKC, ERK and AKT) activated in c643-OPNa and 8505c-OPNa cells and TPC1 cells transfected with siRNA targeting OPN siRNA scrambled negative control.

CONCLUSIONS

These data suggest important roles of $\alpha v\beta 3$ integrin expression and PKC phosphorylation in the context of OPNa overexpression in TC cell lines, further improving our understanding of TC progression, which could contribute to future advances in strategies to propose new therapeutic approaches.

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