EXPRESSION LEVELS OF OSTEOPONTIN AND P53 ISOFORMS PATTERNS IN ENDOMETRIUM CARCINOMA CELL LINES

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INTRODUCTION

Total osteopontin (OPN) is overexpressed in endometrium carcinoma (EC) and modifications at modifications at TP53 gene correspond to one of the major genetic alterations in these tumors. Althought total OPN expression has been correlated to p53 expression, no data is avaliable regarding OPN splice variants and their association to p53 isoforms expression. OPN has three splicing isoforms (OPN-SI), named OPNa, OPNb and OPNc, while p53 has at least 12 variants, such as P53, P53 β, P53 γ, Δ40p53, Δ133p53 and Δ160p53. We aimed to evaluate the expression profile of OPN, p53 isoforms in endometrial tumoral and non tumoral cells.
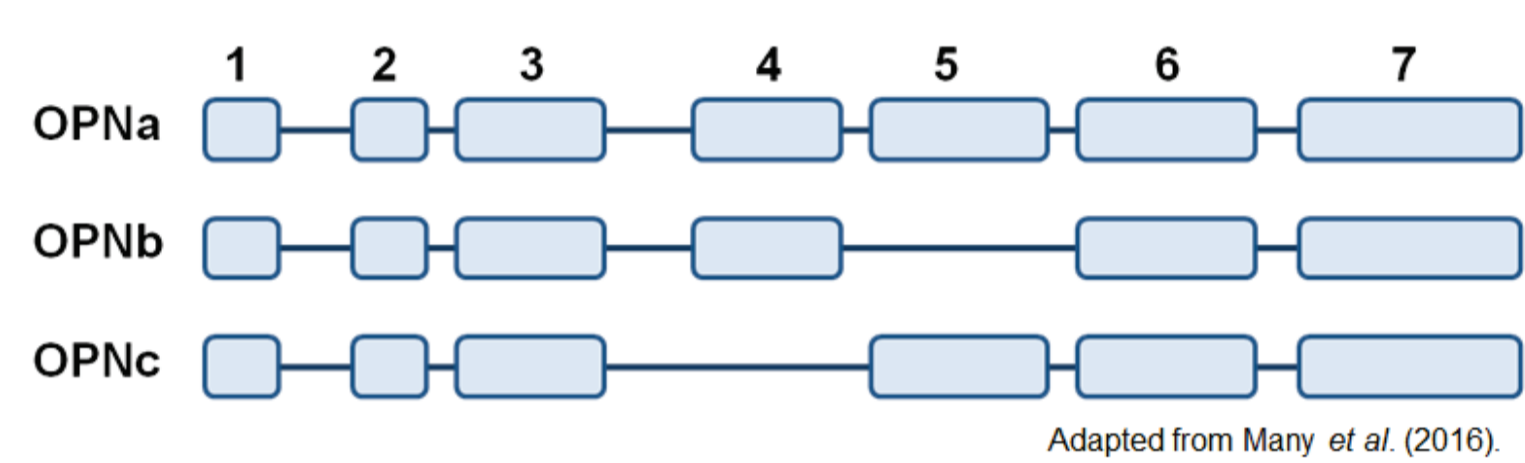

Figure 1: Osteopontin splicing isoforms. OPNa isoform is the complete splice variant, while OPNb does not contain exon 5 OPN and lacks exon 4.
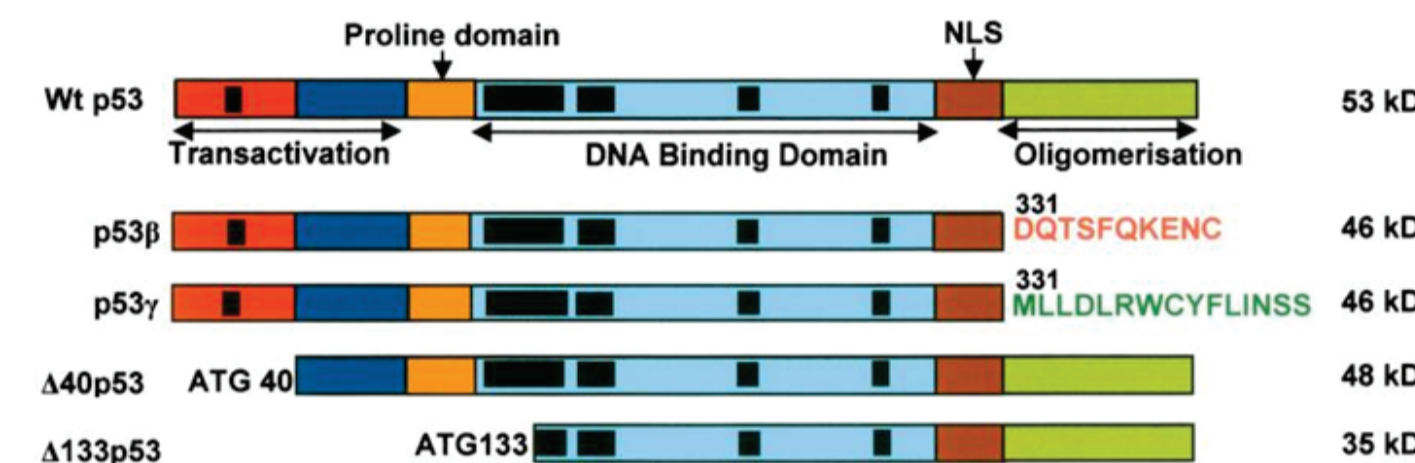

Figure 2: Structure of p53 protein isoforms in humans. Full lenght variant contains the transactivation domain, the proline-rich domain, the DNA binding domain, the nuclear localization signal (NLS) and oligomerization domain. In p53 isoforms, some of these regions has been lost or altered, modifying protein structure.

OBJECTIVES

- Characterize the expression levels of OPN and p53 isoforms in EC and non-tumoral endometrial cell lines.
- Examine the correlation between the expression of P53 and OPN isoforms with the characteristics of the different cell lines tested.
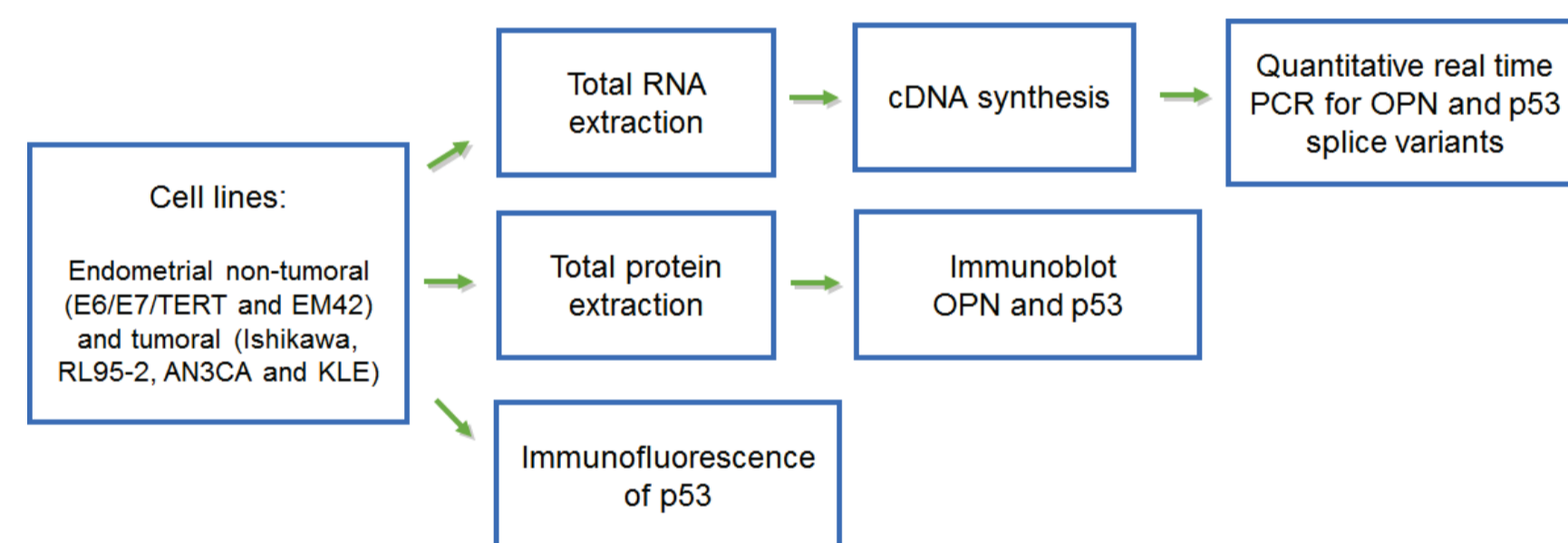
METHODOLOGY

Figure 3: Overall methodology approach to analyze OPN and p53 isoforms expression. In EC cells. These isoforms have been analyzed by quantitative real time PCR, immunoblot and immunofluorescence. Cell lines were cultured in DMEM medium, KLE in whch whose DMEM/F12 mediu has been used. Total RNA was extract using Qiagen mini kit and cDNA synthesis was done with Superscript II First-Strand Synthesis system. Then, cDNa samples were analyzed by quantitative real time PCR. Immunoblot have been performed using total protein extracts, and O-17 anti-total OPN antibody, DO1 anti-p53 antibody. Immunofluorescence assays were performed in AN3CA and E6/E7/TERT cells, using DO1 anti-p53 antibody.

Table 1: Cell line features

Endometrial cell lines	Subtype/Grade
E6/E7/TERT	Non-tumoral
EM42	Non-tumoral
Ishikawa	Type I / G1
RL95-2	Type I / G2
AN3CA	Type I / G3*
KLE	Type II / G3

* Contradictory information, which may be Type I / G3 or Type II / G3

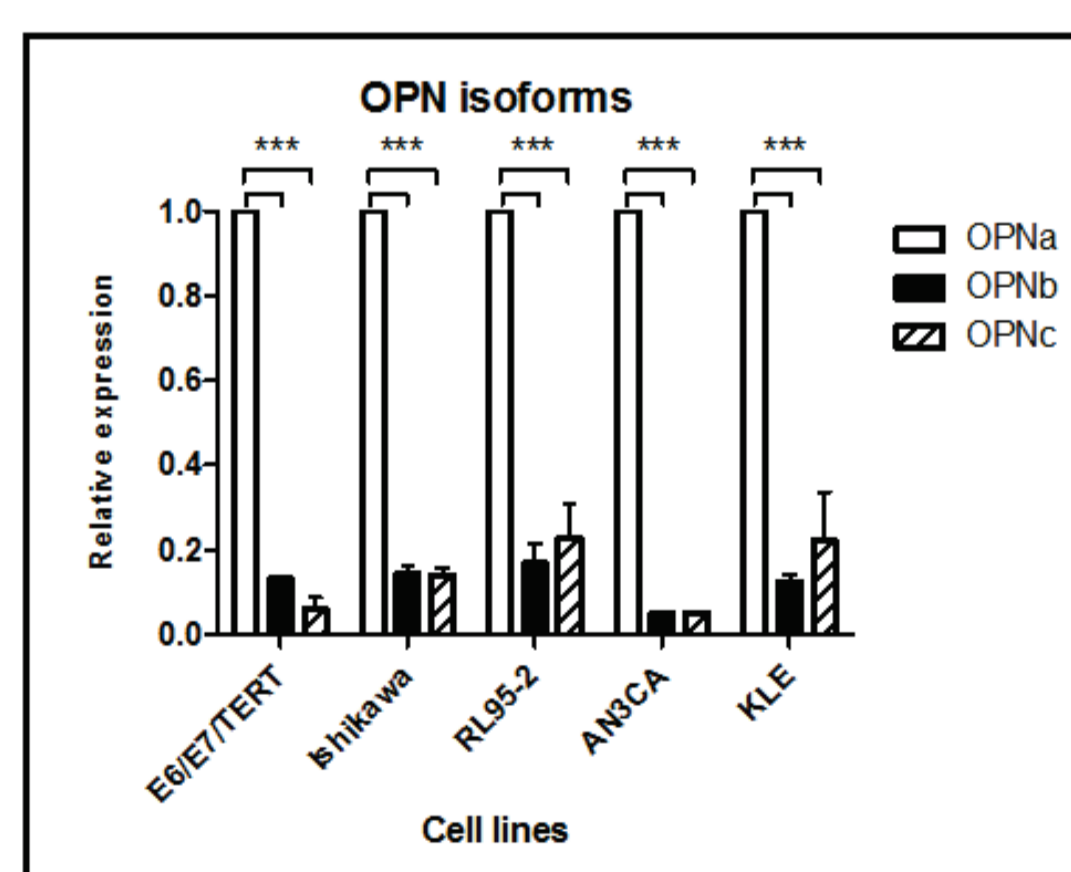Adapted from Bokhari et al. (2016).

RESULTS

Figure 4: OPN isoforms expression levels in EC and non-tumoral E6/E7/TERT cell lines. The transcriptional levels of OPN isoforms were analyzed by qRT-PCR, using isoform-specifc oligonucleotides. GAPDH was used as constitutive expression control. The results presented were performed in duplicate in 3 independent assays, using the OPNa isoform as the reference sample to calculate relative expression levels (reference value = 1). We found that OPNa isoform is expressed in higher levels in relation to OPNb and OPNc in all tested cell lines. The p values refer to the comparison between the transcriptional level of in relation to OPNb or OPNc isoforms. *** P <0.001.
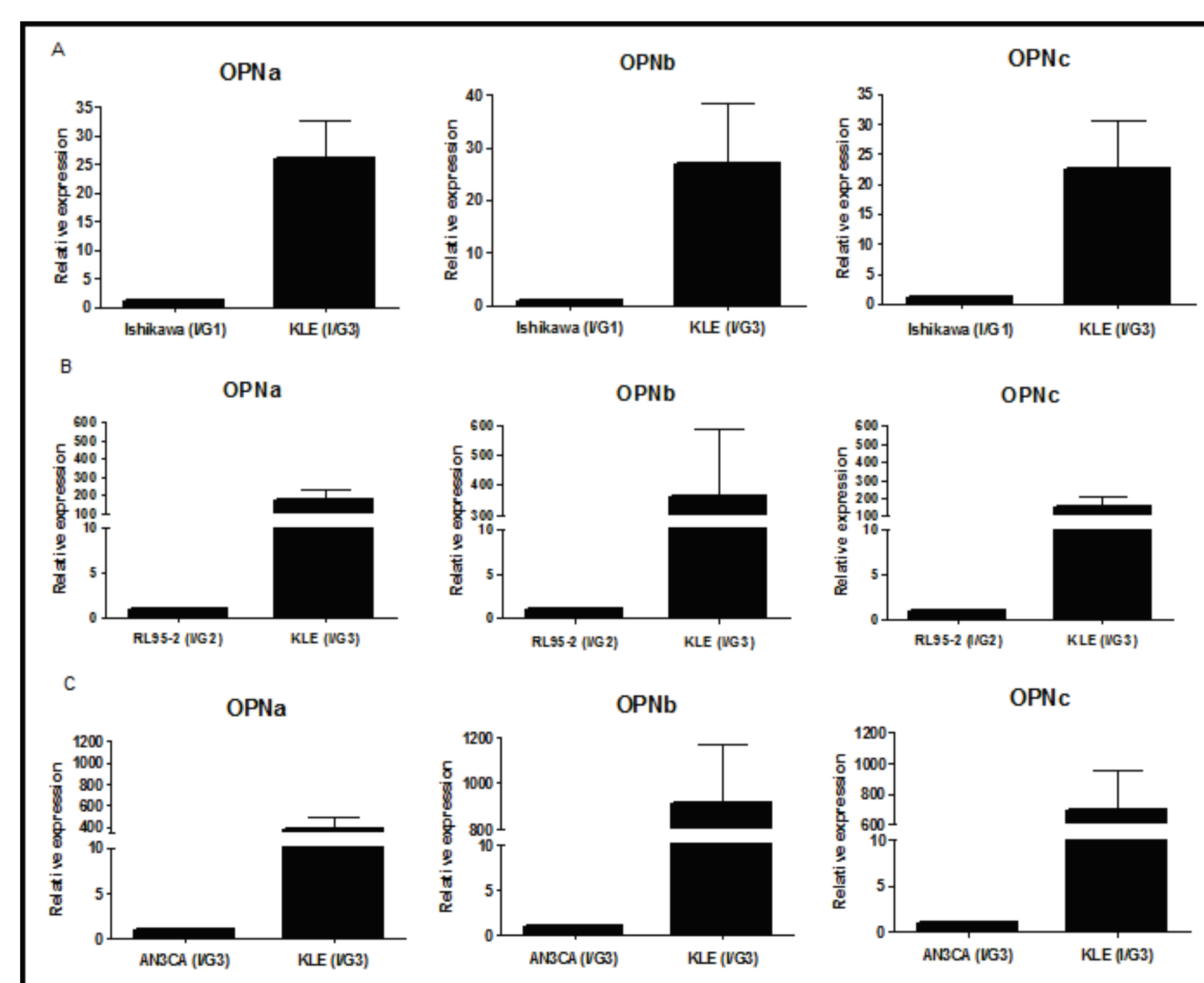

Figure 5: OPN isoforms expression levels between EC cell lines. The transcriptional levels of each OPN isoform was analyzed by qRT-PCR, using isoform-specifc oligonucleotides. GAPDH was used as constitutive expression control. We compared the expression levels of each OPN splice variant between KLE and three additional cell lines, representing distinct EC tumor grades. The expression of each OPN variant in KLE was compared to was compared to Ishikawa (A), RL95-2 (B) and AN3CA (C), which were used as reference samples. We found that KLE cell line had higher expression levels of OPN isoforms, even when compared to another G3, AN3CA. The results presented were performed in duplicate in 3 independent assays.
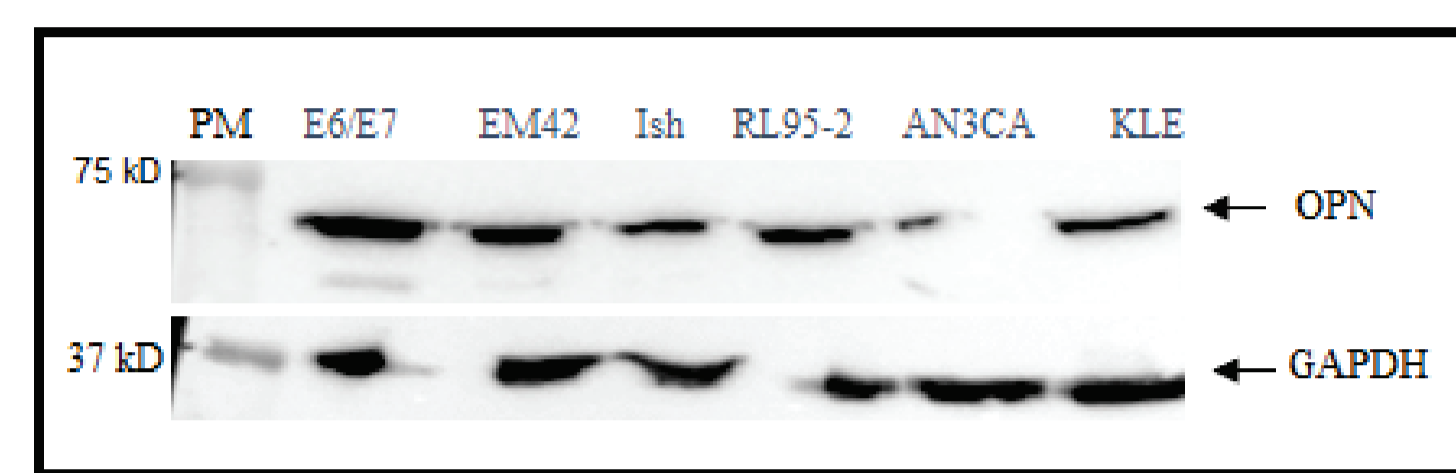

Figure 6: Analysis of total OPN protein expression by immunoblot in EC and endometrial non-tumoral cell lines. The expression of total OPN was analyzed using the O-17 anti-total OPN rabbit antibody. At the protein level, both EC and endometrial non-tumoral cell lines express total OPN. However, we can not assert which isoform is being mostly detected using this assay. The endogenous total OPN protein expression was analyzed using 60μg of each protein extract. GAPDH expression was used as an endogenous constitutive control.
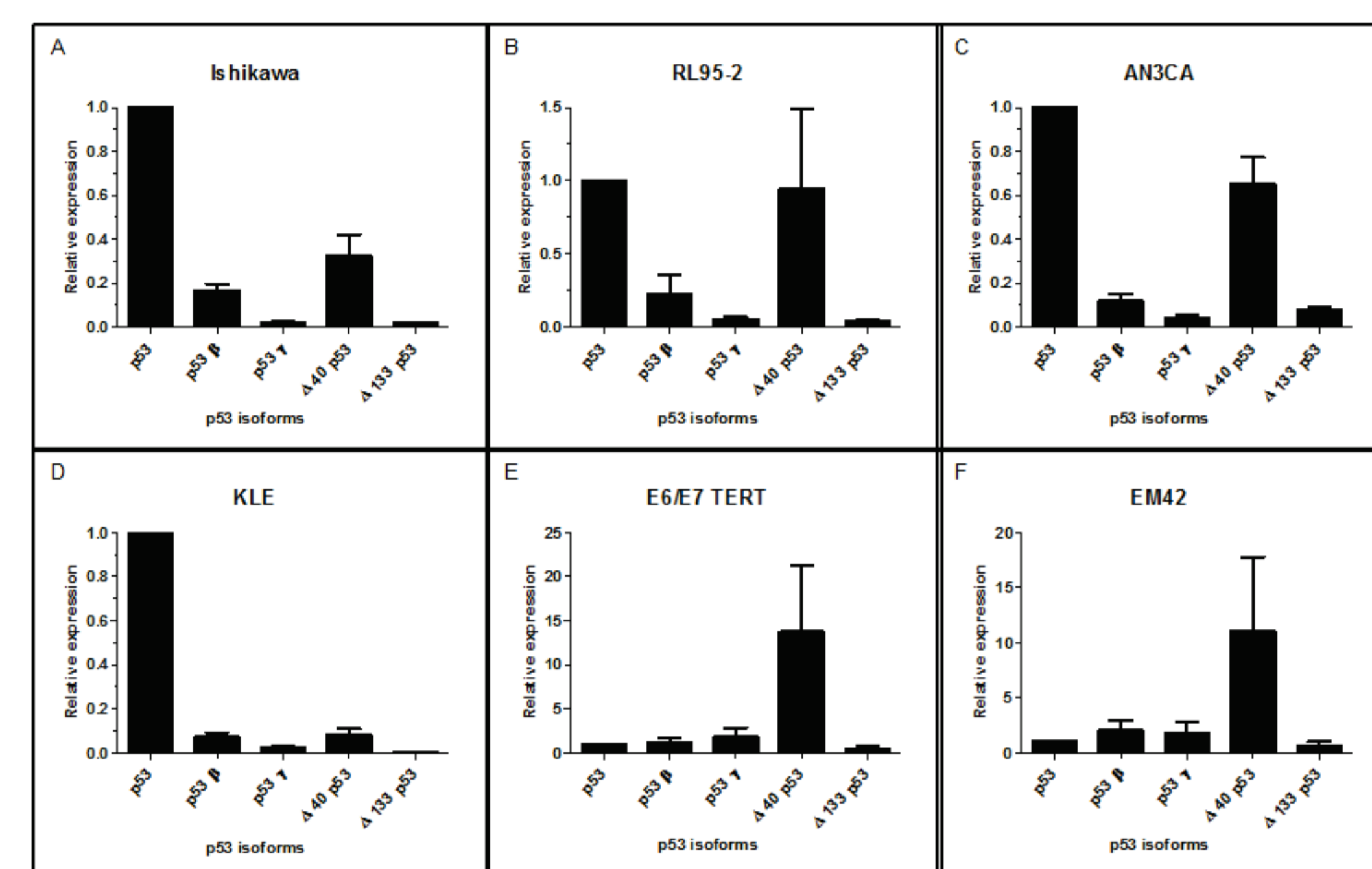

Figure 7: p53 isoforms expression levels in EC and non-tumoral cell lines. P53 isoforms transcriptional levels were analyzed by qRT-PCR using isoform-specific oligonucleotides and β-actin was used as the constitutive gene. The p53 isoform relative expression levels were analyzed respectively, in Ishikawa (A), RL95-2 (B), AN3CA (C), KLE (D), E6 / E7 / TERT (E) and EM42 (F) cells, using full length p53 as the reference sample for relative expression calculation. In tested tumor cell lines (Ishikawa, RL95-2, AN3CA and KLE), the full lenght p53 isoform is overexpressed in relation to the others isoforms. However, in EC subtype I representative cell lines (Ishikawa, RL-95-2 and AN3CA), the Δ40p53 isoform is also expressed in higher levels (A, B and C). Conversely, Δ40p53 isoform is predominant isoform in endometrial non-tumoral cell lines tested (E and F). The results presented were performed in duplicate in 3 independent assays.
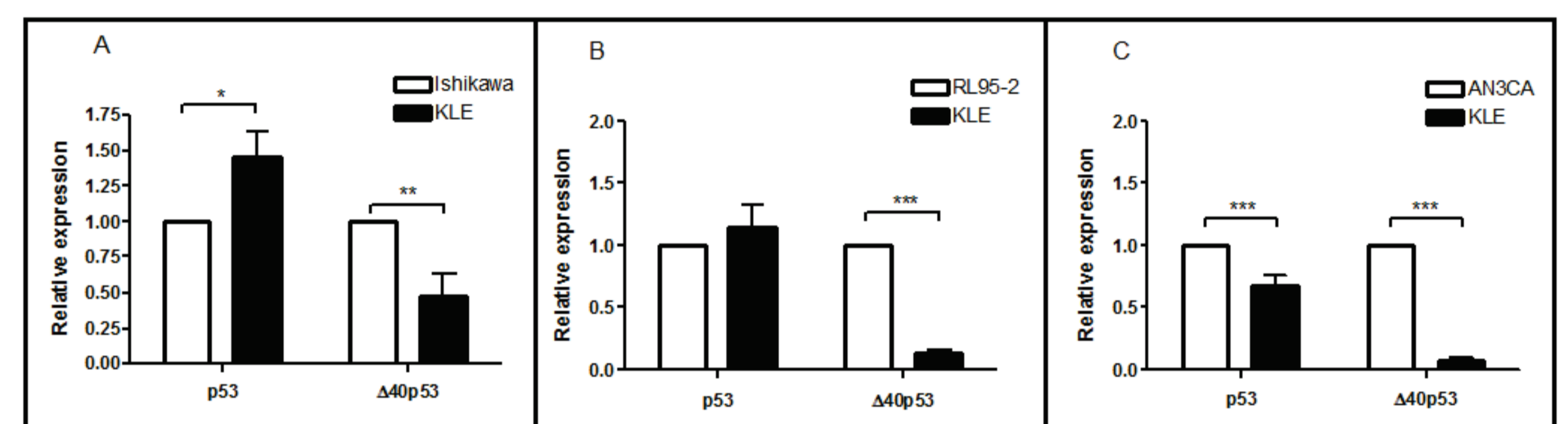

Figure 8: Expression level of p53 and Δ40p53 isoforms in EC cell lines. The transcriptional levels of p53 and Δ40p53 isoforms was analyzed by qRT-PCR, using isoform-specific oligonucleotides. β-actin was used as the constitutive gene. We compared the expression levels of each p53 isoform between Ishikawa(A), RL95-2 (B) and AN3CA (C) cell lines as the reference sample to KLE cell line. We observed that the KLE cell line has a higher transcriptional expression level of full lenght p53 than Ishikawa and RL95-2 cell lines. However, a lower transcriptional expression level when compared to the AN3CA cell line. The Δ40p53 isoform shows a lower transcriptional expression level in KLE when compared to Ishikawa, RL95-2 and AN3CA cell lines. The results presented were performed in duplicate in 3 independent assays. * P <0.05, ** P <0.01 and *** P <0.001.
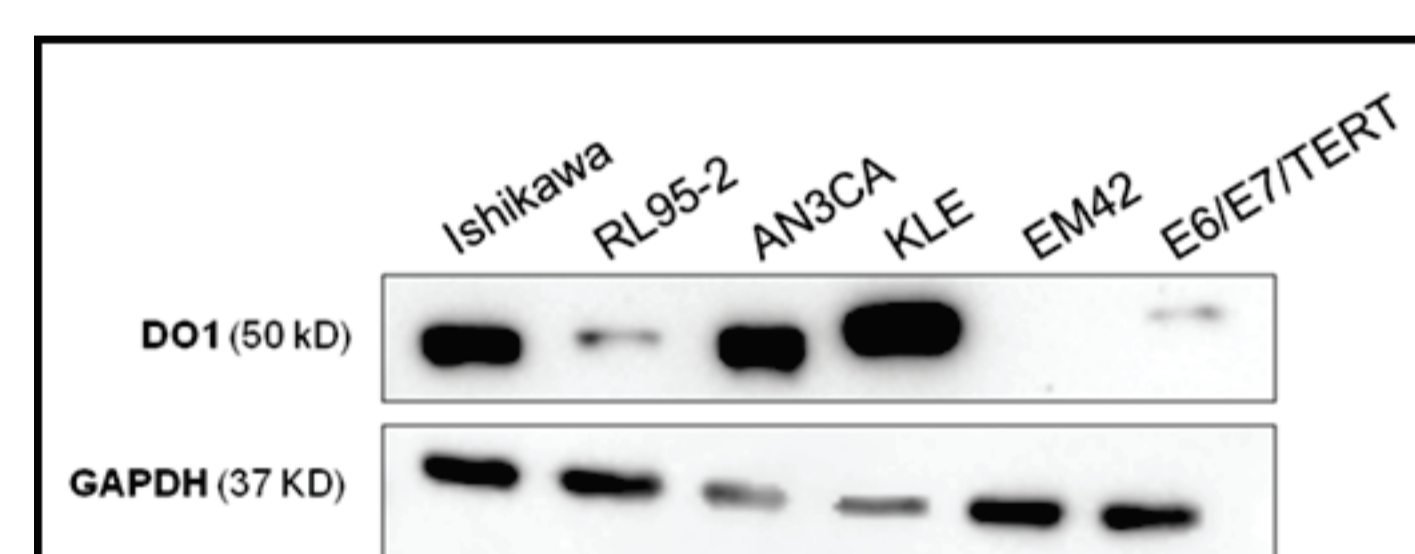

Figure 9: Full lenght p53 isoform protein expression in tumor and non-tumoral endometrial cell lines. The endogenous protein expression of full lenght p53 was analyzed using the DO-1 anti-p53 antibody, which recognizes an epitope located between amino acids 20-25 of full length p53. Thus, this antibody does not recognize the Δ133p53 or Δ40p53 truncated isoforms in the p53 N-terminal portion. GAPDH expression was used as an endogenous constitutive control. We observed that full length p53 display higher protein expression levels in all tested EC cell lines in relation to non-tumoral cells EM42 and E6/E7/TERT.
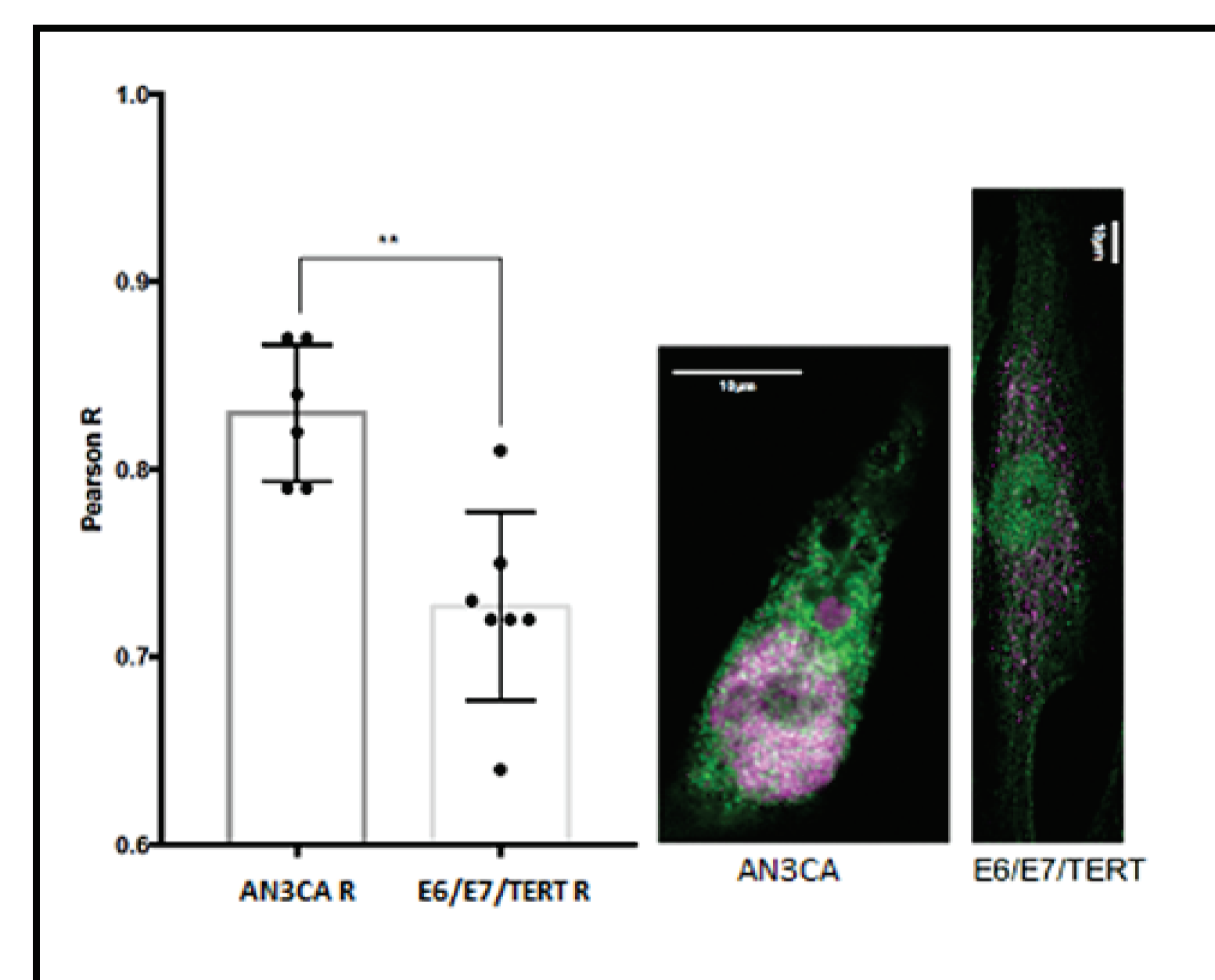

Figure 10: Immunofluorescence analysis of full lenght p53 and protein oligomers expression in tumor (AN3CA) and non-tumoral (E6/E7/TERT) endometrial cells. We foun by The Person correlation analysis that full length p53, as detected by DO-1 antibody (pink staining) exhibts a higher co-localization index to total protein oligomers (detected by A11 antibody, shown green staining) in AN3CA endometrium carbinoma cells than in non-tumoral cells E6/E7/TERT. In tumor cell lines total p53 co-aggregation to oligomers was mostly nuclear, as opposed to that observed in E6/E7/TERT, which was mainly cytoplasmic.

CONCLUSIONS

These data provide early evidence that both OPN and p53 full-leght variants are the major isoforms in both tumor and non-tumoral cells, but their splice variants present differential expression accoding to EC cell differentiation. Further work should investigate the impact of OPNa overerexpression over p53 isoforms and their putative cellular and molecular roles on modulating p53 tumor suppressive roles and aggregation status.

Projeto Gráfico: Setor de Edição e Informação Técnico-Científica / INCA