4th Metabolism and Cancer Symposium

Protein kinase C regulates fatty acid metabolism in breast cancer cells

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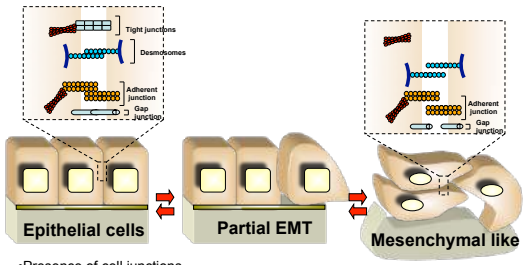
INTRODUCTION An epithelial-mesenchymal transition (EMT) is a biologic process that allows a polarized epithelial cell, which normally interacts with basement membrane via its basal surface, to undergo multiple biochemical changes that enable it to assume a mesenchymal cell phenotype, which includes enhanced migratory capacity, invasiveness, elevated resistance to apoptosis, and greatly increased production of ECM components (Kalluri et al., 2003).



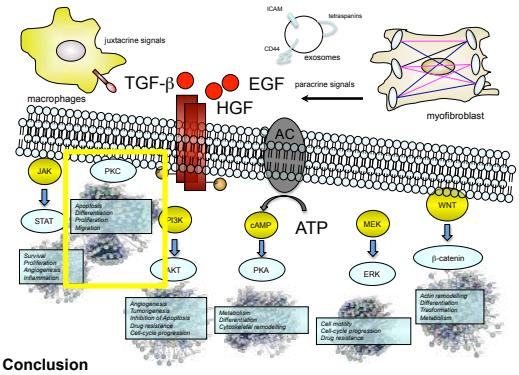
- Presence of cell junctions
- Apicobasal polarity
- Limited migratory ability
- Expression of epithelial markers
  - E-cadherin
  - Cytokeratins
  - Occludins

- Absence of stable cell junctions
- Anterior/posterior polarity
- Increased matrix degradation
- Expression of mesenchymal markers
  - N-cadherin
  - Fibronectin
  - Laminin

AIM:

To investigate the role of protein kinase C in the regulation of lipid metabolism in breast cancer

The process of EMT involves significant changes in multiple cellular processes including lipid metabolism. In breast cancer cells, protein kinase (PKC) has been shown to trigger malignant progression. It is conceivable that PKC can contribute to cancer plasticity by regulating lipid metabolism. In this work, we investigated the effects of the phorbol ester 12-myristate 13-acetate (PMA)-mediated activation of PKC in MDA-231 cells.



Conclusion

Here, we demonstrate that PKC activation drives the expression of metabolic enzymes involved in fatty acids mobilization from triacylglycerols (TAG) and lipid utilization in the mitochondria through fatty acid oxidation (FAO).

PMA-treatment of MDA-231 cells induced a rapid activation of PKC as revealed by western blot (Figure 4A). Using LC-MS/MS, we observed a distinct proteome expression pattern in PMA-treated cells relative to control cells (Figure 4B). In triple negative cells, we report that PKC regulates intracellular fatty acid metabolism through the up-regulation of N-Myc Downstream Regulated 1 (NDRG1) and acyl-coenzyme A (CoA) synthetase long chain family member 3 (ACSL3) (Figure 4C). This was correlated with higher rate of [^{14}C]CO2 production, the end product of labeled palmitate oxidation, in PMA-treated cells with respect to MDA-231 control cells (Figure 4D). To further assess the contribution of PKC in FAO, we measured [^{14}C]CO2 production after MDA-231 cells treatment with the PKC inhibitor Ro 31-8220. We found that [^{14}C]CO2 production was lower in Ro 31-8220 treated cells (Figure 4D). *In vivo*, NDRG1 is highly expressed in triple-negative breast cancers (TNBCs) as compared to hormone receptors-positive and human epidermal growth factor 2 receptor–negative or positive breast cancer (Figure 4E).

Overall, these findings demonstrate the critical role of PKC in supporting the increased bioenergetic reliance on FAO of TNBC.

Previous work: A specific lipid metabolic profile is associated with the EMTprogram.

Here, we studied epithelial and mesenchymal breast cancer cells by proteomic and lipidomic approaches and identified significant differences that characterised these models concerning specific metabolic enzymes and metabolites including fatty acids and phospholipids (Figures 1 and 2). Higher levels of monounsaturated fatty acids together with increased expression of enzymes of de novo fatty acid synthesis is the distinct signature of epithelial with respect to mesenchymal cells that, on the contrary, show reduced lipogenesis, higher polyunsaturated fatty acids level and increased expression of genes involved in the triglyceride (TAG) synthesis and lipid droplets formation. In the mesenchymal model, the diacylglycerol acyltransferase (DGAT)-1 appears to be the major enzyme involved in TAG synthesis and inhibition of DGAT1, but not DGAT2, drastically reduces the incorporation of labeled palmitate into TAG (Figure 3) (Biochim Biophys Acta Mol Cell Biol Lipids. 2019 Mar; 1864(3):344-357).

Figure 1. LC-MS/MS analysis of epithelial and mesenchymal models

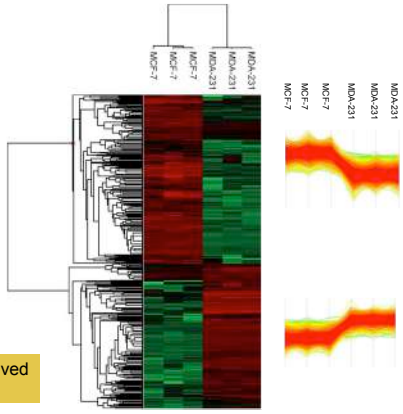


Figure 3. Differential expression and activity of enzymes involved in lipid metabolism between MCF-7 and MDA-231 cells

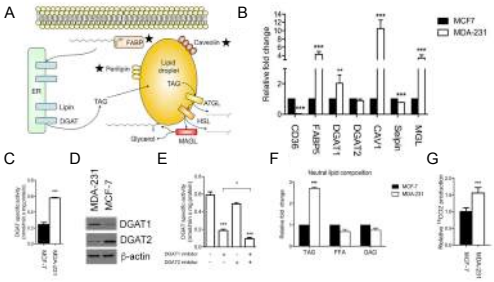


Figure 2. Lipidomic analysis of MCF-7 and MDA-231 cells

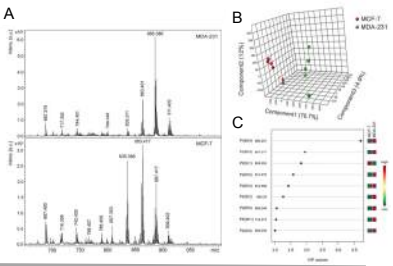
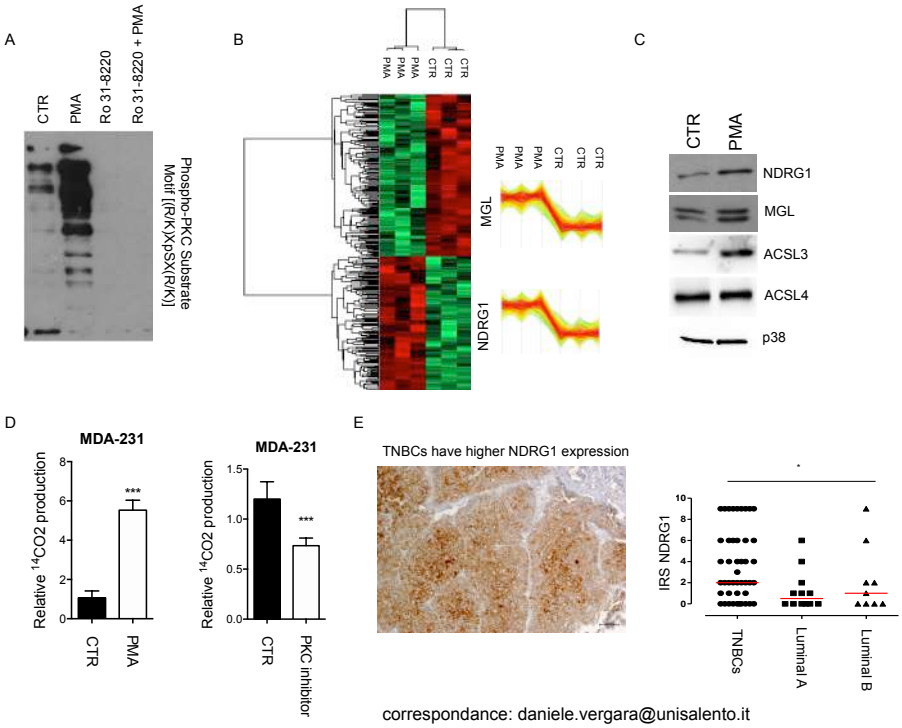


Figure 4. PKC activation modulates lipid metabolism in MDA-231 cells



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