Hypoxia regulates ANXA1 expression to support prostate cancer cell invasion and aggressiveness

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Prostate cancer (PCa) is one of the most widespread tumors and the sixth leading cause of cancer-related death in men of Western industrialized countries. PCa onset and development are generally androgen-dependent but in the course of time the tumor tends to convert into a castration-resistant disease that remains largely incurable. This transition to castration resistant PCa (CRPC) is supposed to originate by several genetic mutations underlying activation of oncogenes and/or inactivation of tumor suppressor genes however, many of the mechanisms essential for PCa progression remain to be clearly defined (Grasso et al., 2012). A number of evidences are accumulating that the tumor microenvironment strongly contributes to the malignant transformation of cancer cells and that hypoxia is one of the environmental feature essential to progression of solid tumors, including PCa. It is well known that, because of a size increase, the inner areas of the tumor mass become gradually hypoxic until enough blood vessels are formed. Hypoxic conditions within tumors purportedly result in improved expression, stability and activity of hypoxia inducible factor 1 (HIF-1) (Kietzmann et al., 2016). Once stabilized and activated, HIF1 regulates genes generally associated with a poor prognosis and with pro-oncogenic processes such as cancer cell spreading and invasion and metastasis formation (Bae et al., 2016). One of the proteins up-regulated in low oxygen conditions by HIF-1 over-expression is annexin A1 (ANXA1) (Liao et al., 2009).

ANXA1 is a 37 kDa protein belonging annexin superfamily of proteins and able to bind membrane phospholipids in a Ca2+-dependent manner. This protein is involved in a wide range of physio-pathological processes, including cancer development (Bizzarro et al., 2015; Belvedere et al., 2016). Its dysregulation in PCa has been reported by numerous studies with controversial results however, the protein seems to be over-expressed in the invasive stages of this tumor (LaTulippe et al., 2002; Taylor et al., 2010; Setlur et al., 2008).

We have previously demonstrated that ANXA1 is able to confer mesenchymal/metastatic features and a more aggressive phenotype in chemo-resistant DU145 PCa cells (Bizzarro et al., 2015). Here we have investigated the mechanism(s) by which ANXA1 promotes PCa progression, utilizing a well characterized in vitro experimental model of cancer development based on the comparison of the three human LNCaP, DU145 and PC3 PCa cells. These cell lines differ in their phenotypes and malignancy levels and reflect the androgen independent progression of PCa: more interestingly, they show dissimilar expression profiles of ANXA1 protein (Hasegawa et al., 2006). Thus, we have analyzed the effects of hypoxic conditions on ANXA1 expression and on the metastatic-associated functional behaviors of transiently ANXA1 knock down and over-expression in PCa cells. We show that ANXA1 was differentially expressed by PCa cell lines in normoxia whereas hypoxic stimuli resulted in a significant increase of protein expression. Additionally, in low oxygen conditions ANXA1 was extensively secreted outside the cells where its binding to formyl peptide receptors (FPRs) induced cell invasion. Loss and gain of function experiments performed by using the RNA

interfering siANXA1 and an ANXA1 over-expressing plasmid (MF-ANXA1), also confirmed the leading role of the protein in modulating LNCaP, DU145 and PC3 cell invasiveness. Finally, ANXA1 played a crucial role in the regulation of cytoskeletal dynamics underlying metastatization process, such as the loss of adhesion molecules and the occurrence of the epithelial to mesenchymal transition (EMT). ANXA1 expression increased inversely to epithelial markers such as E-cadherin and cytokeratins 8 and 18 (CKs) and proportionally to mesenchymal ones such as vimentin, ezrin and moesin.

The results from this study suggest that hypoxia may modify the expression and the localization of ANXA1 which in turn promotes hypoxia-related cell invasion by regulating expression and activity of proteins involved in EMT, cell shape, adhesion and motility. The present data suggest that ANXA1 may serve as a potential target for therapeutic interventions for metastatic PCa.

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