P35 Combined targeting of cholesterol and steroid biosynthesis effectively inhibits enzalutamide resistant prostate cancer cells and 3-dimensional spheroid growth

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Introduction & Objectives: Although antiandrogens have certainly revolutionized treatment of PCa patients, progression to castration resistance (CRPC) and the frequent occurrence of therapy resistances are still challenging for an effective therapy of advanced PCa. We have previously shown that co-culture spheroids consisting of cancer-associated fibroblast (CAFs) and PCa cells become resistant to the antiandrogen enzalutamide. In the present study, we investigated gene expression changes, which occur in PCa cells upon 3-dimensional (3D) co-culture with CAFs to identify novel mechanisms for enzalutamide resistance.

Materials & Methods: Spheroids were established in hanging drop plates by seeding PCa cells (LNCaP, DuCaP) alone or together with CAFs. Gene expression changes were identified through Illumina microarrays. Real-time PCR, Western blotting and immunohistochemistry were used to validate expression of selected targets. Cell viability was assessed by a colorimetric assay.

Results: Microarray analysis revealed that PCa cells exhibited a typical gene expression profile in 3D culture, which was hallmarked by upregulation of cell adhesion and ECM-receptor interaction pathways and downregulation of cell cycle and DNA replication. Similarly, CAFs presented a low proliferative activity with a strong inflammatory phenotype and activation of fatty acid metabolism in 3D culture. In co-culture with CAFs, particularly cholesterol and steroid biosynthesis pathways were significantly upregulated in PCa cells. Among the most significantly upregulated genes within these pathways were 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2 (HMGCS2) and aldo-keto reductase family 1 member C3 (AKR1C3), which were also significantly increased in human PCa specimens compared to benign tissue. Stable knockdown of HMGCS2 significantly inhibited PCa spheroid growth and sensitized the cells to enzalutamide, whereas ectopic expression of HMGCS2 stimulated spheroid growth and made the cells more resistant to enzalutamide, suggesting a critical role of this enzyme in PCa. Blocking cholesterol synthesis via simvastatin was even more effective and inhibited enzalutamide resistant LN/CAF and Du/CAF co-culture spheroids as well as a panel of enzalutamide (DuCaPEnzaR, LNCaPablEnzaR, CWR22Rv1) and castration resistant (LNCaPabl) PCa cell lines. A specific AKR1C3 inhibitor was also able to counteract enzalutamide resistance and even potentiated the anti-proliferative effects of simvastatin.

Conclusions: In the present study we show that upregulation of cholesterol and steroid biosynthesis in PCa cells through co-culture with CAFs has a strong impact on enzalutamide resistance. Hence, targeting these pathways through simvastatin and/or AKR1C3 inhibitors could be an adjuvant therapy approach in patients with therapy-resistant PCa.