

Title: Autophagy as an adaptive survival pathway in androgen-mediated MYC suppression in prostate cancer.

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Background: Prostate cancer is the most diagnosed cancer in men, with many patients progressing to lethal castration-resistant prostate cancer (CRPC) after androgen deprivation therapy or second-generation androgen receptor pathway inhibitors. Bipolar androgen therapy (BAT), which alternates supraphysiological androgen (SPA) with castration levels, achieves objective and PSA50 responses in 30–50% of CRPC patients, including those resistant to prior ARPIs. The anti-tumor effects of SPA are linked to the suppression of the oncogene MYC. However, resistance to treatment often develops, and the adaptive mechanisms that enable tumor cells to survive MYC suppression remain poorly understood. Given that MYC repression can activate autophagy and lysosomal biogenesis in cancer, we hypothesize that SPA-induced MYC suppression promotes autophagy as a survival mechanism, limiting the durable response to BAT.

Methods: We treated prostate tumor cells with SPA and assessed MYC protein/transcript by immunoblot and qPCR. Autophagic flux was measured using LC3B-II/I conversion, p62 turnover, and hydroxychloroquine (HCQ)-based flux discrimination. MYC necessity/sufficiency for autophagy induction was tested via MYC restoration in SPA-treated cells and via MYC suppression (CRISPR knockout, JQ1) in the absence of SPA. Cytoprotective function of autophagy was tested by combining SPA with the autophagy inhibitor HCQ and assessing viability and clonogenic growth. A prostate xenograft model (SkCaP-1R) tracked MYC, LC3B-II, and p62 dynamics during the emergence of SPA resistance, and bulk RNA-seq characterized autophagy/lysosomal gene programs over the course of treatment.

Results: SPA suppressed MYC protein and transcript levels in a dose- and duration-dependent manner selectively in high-AR cell lines, with no effect in AR-negative controls. This MYC suppression was accompanied by increased LC3B puncta and enhanced autophagic flux. With prolonged treatment, tumor cells develop resistance to SPA, characterized by AR downregulation and MYC re-expression, and by transiently rising signatures of autophagy and lysosomal biogenesis before returning to baseline as resistance emerges, demonstrating a reciprocal relationship between MYC and autophagy induction. Direct MYC knockdown (CRISPR, JQ1) was sufficient to induce autophagy independent of SPA, while constitutive MYC overexpression blunted both basal and SPA-induced autophagy, establishing MYC suppression as a proximal driver of this response. Pharmacologic inhibition of autophagy with HCQ significantly reduced viability and clonogenic survival of SPA-treated cells and was associated with enhanced MYC suppression in combination-treated cells, consistent with autophagy functioning as a cytoprotective, pro-survival pathway during MYC loss.

Conclusion: Our findings support a model in which AR-driven MYC suppression during SPA exposure derepresses autophagy as an adaptive survival mechanism that contributes to therapeutic resistance in BAT-treated CRPC. Autophagy inhibition impairs cell survival and outgrowth and prolongs MYC suppression, suggesting combined SPA and autophagy blockade as a rational strategy to deepen and extend responses to bipolar androgen therapy.

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