Understanding the molecular mechanisms promoting therapy resistance is important. Previously, we reported that VEGFC can promote cancer cell survival during stress via interaction with its receptor NRP2. While examining the molecular mechanisms involved in this survival, we performed a microarray study in which we identified two genes, WDFY1 and LAMP2, which have been suggested to function in autophagy. Our subsequent studies further confirmed the regulation of autophagy by the VEGFC-NRP2 axis in cancer during starvation- and chemotherapy-induced stress. We are currently in the process of determining the mechanism(s) through which WDFY1 and LAMP2 control autophagy; however, we did observe an increase in MTOR complex 1 (MTORC1) activity after the depletion of the VEGFC-NRP2 axis. It would therefore be interesting to study whether WDFY1 and LAMP2 can influence MTORC1 activity and regulate autophagy. Taken together, our data suggest that targeting the VEGFC-NRP2 axis in combination with chemotherapy could be an effective treatment for advanced cancers.

The ability of tumor cells to survive and recur despite stress is a major impediment to effective cancer therapy. Hence, it is important to understand the molecular mechanisms that promote resistance to therapy in cancer cells. One member of the vascular endothelial growth factor (VEGF) family, VEGFC, upon binding to its non-tyrosine kinase receptor, NRP2, in cancer cells promotes their survival in the presence of stress. To understand the molecular mechanisms responsible for this survival-promoting function of this axis, we performed a microarray study following the depletion of either VEGFC or NRP2 in prostate cancer cells and identified LAMP2 and WDFY1, which both encode proteins suggested to function in autophagy, a stress-induced self-digestion process that has been demonstrated to promote cancer cell survival, tumor dormancy, and subsequent progression and metastasis.

To characterize the effect of VEGFC or NRP2 depletion in starved cells, we monitored the levels of LC3-II, the lipidated form of LC3, which is incorporated into the forming autophagosome membrane. Initially, we observed that LC3-II levels increase following depletion of VEGFC or NRP2. The observed increase in LC3-II levels could be attributed to either an increase in autophagic trafficking, or to a decrease in autophagic degradation, as LC3-II is degraded by the lysosome. Therefore, we performed autophagic flux assays in which a subset of cells was treated with bafilomycin A1 (BAFA1) to prevent autophagosome-lysosome fusion. The fold-change in LC3-II levels was then determined by comparing the level of LC3-II in BAFA1-treated cells compared with that in nontreated cells. Here, we observed a decrease in the fold-change of LC3-II in VEGFC- or NRP2-depleted cells compared with that in nontreated cells. Here, we observed a decrease in the fold-change of LC3-II in VEGFC- or NRP2-depleted cells compared with controls; indicating that the depletion of the VEGFC-NRP2 axis leads to dysregulated autophagic degradation.

We reasoned that VEGFC-NRP2-driven autophagy could serve as a mechanism through which cancer cells could evade chemotherapy-induced death, thereby promoting tumor cell survival.
Therefore, we repeated our autophagic flux experiments in prostate cancer (PCa) cells treated with docetaxel, the chemotherapeutic agent used to treat advanced-stage metastatic prostate cancer. In this work, we found that docetaxel treatment activated autophagic trafficking, which was abrogated through the depletion of either VEGFC or NRP2. From these data, we concluded that the VEGFC-NRP2 axis promoted chemotherapy-induced autophagy. We also confirmed this visually using PCa cells stably expressing mCherry-GFP-LC3 depleted of VEGFC or NRP2 and treated them with docetaxel. Following autophagy initiation, yellow and green puncta indicative of autophagosome formation and red puncta corresponding to autolysosomes were visible in control cells. In contrast, in VEGFC- or NRP2-depleted cells, only a diffuse green staining or green puncta were observed confirming that the maturation of autophagosomes into autolysosomes did not occur. Furthermore, when we calculated the ratio of green puncta to red puncta, we found a decrease in red puncta formation in VEGFC- or NRP2-depleted cells. Combined, these data validated the findings of our autophagic flux experiments. We observed similar results in the CaPan-1 pancreatic cancer cells. CaPan-1 cells were treated with gemcitabine, a chemotherapeutic drug used to treat metastatic pancreatic cancer. These results indicate that the upregulation of the VEGFC-NRP2 axis during chemotherapy treatment provides a generalized mechanism though which different cancers can avoid death. Interestingly, this function is specific for VEGFC, as we did not observe similar autophagy regulation when we knocked down VEGFA in cancer cells.

We previously observed increases in LAMP2 and WDFY1 levels following the depletion of the VEGFC-NRP2 axis. The inhibition of autophagy via BAFA1 treatment also led to an increase in LAMP2 and WDFY1 levels. When we examined whether increased LAMP2 and WDFY1 levels influenced cancer cell survival during chemotherapeutic stress, we found enhanced cell viability in cells co-depleted of either VEGFC or NRP2 and WDFY1 compared with cells depleted solely of VEGFC or NRP2. We also detected an increase in cell viability following co-depletion of LAMP2 and the VEGFC-NRP2 axis. Based upon these results, we concluded that LAMP2 and WDFY1 upregulation following the blockade of autophagy promotes cell death.

Although the increase in WDFY1 and LAMP2 following VEGFC-NRP2 depletion can induce cell death, their role in promoting autophagy downstream of the VEGFC-NRP2 axis is still unclear. We previously demonstrated that the VEGFC axis maintains MTORC2 activity which is upstream of AKT, while the downstream mediator MTORC1 remained inactive in PCa cells during oxidative stress. We therefore hypothesized that the VEGFC-NRP2 axis inhibits MTORC1 activity to promote autophagy during stress. Following the depletion of either VEGFC or NRP2 in cancer cells, we found significantly increased levels of the phosphor-S6K1 indicating the activation of MTORC1. The autophagic blockade could be reversed by treating cells with rapamycin, an MTORC1 inhibitor. Combined, our results indicate that the VEGFC-NRP2 axis promotes autophagy and subsequent tumor cell survival via the downregulation of MTORC1 activity when chemotherapeutic stress is present.

Overall, our data suggest potential therapeutic significance of targeting the VEGFC-NRP2 axis in combination with established chemotherapy in advanced cancers. In the future we will determine the temporal and spatial roles WDFY1 and LAMP2 play in the control of autophagy and whether they influence MTORC1 activity in the absence of VEGFC-NRP2.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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