Editorial

Notch versus the proteasome: what is the target of γ-secretase inhibitor-I?

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Abstract

γ-Secretase inhibitors are new anti-cancer agents targeting Notch signaling. Their specificity for Notch is as yet unclear. Han and colleagues investigated the effects of Z-LeuLeuNleu-CHO on growth of breast cancer cells. The results demonstrated a reduction in cell viability primarily via proteasome inhibition independent of Notch activity. Currently, γ-secretase inhibitors in clinical trials are structurally distinct from Z-LeuLeuNleu-CHO. Their effects on the proteasome are yet to be determined. However, findings from Han and colleagues pose two critical questions: Is the level of proteasomal activity in breast tumors the driving force for growth? What does the Notch pathway contribute to this growth?

Breast cancer continues to be the second leading cause of cancer-related deaths among women. In a recent article in *Breast Cancer Research*, Han and colleagues investigated the effects of γ-secretase inhibitor (GSI)-I on growth of genetically different breast cancer cells [1].

Along with GSIs, current therapeutic strategies are increasingly promising – particularly those targeted at ErbB-2 (HER2$^+$)-positive or estrogen receptor alpha (ERα$^+$)-positive breast tumors. Triple-negative (HER2-negative, ERα-negative, and progesterone receptor-negative) or basal-like breast cancers lack targeted treatment, demonstrating the highest mortality rate and overall reduced disease-free survival [2]. Emerging treatment approaches to help increase overall patient survival lie in targeting novel pathways for the specific subtypes of breast cancer. New chemotherapeutics aim at targeting the Notch signaling pathway, one of the developmental cell-to-cell contact transmembrane proteins involved in cell fate determination, in cell differentiation, in cell proliferation, and possibly in tumor-initiating cells [3]. Notch-1 and its ligand, Jagged-1, have been shown to be co-overexpressed in breast cancer and to be correlated with the poorest overall survival [4], and thus the signaling pathway has surfaced as a potential therapeutic target.

The Notch pathway has a critical cleavage step involving a complex of four proteins called the γ-secretase complex. Enzymatic cleavage of Notch by the γ-secretase complex, the third proteolytic cleavage in the pathway, is essential for the formation of the active intracellular Notch domain: this is therefore a desirable step for targeted inhibition. GSIs are still in their infancy, leaving desired mechanistic effects necessary to elucidate.

In the previous issue of *Breast Cancer Research*, Han and colleagues investigated the targeted effects of GSI-I [1]. Their findings using GSI-I, a drug aimed at inhibiting the Notch pathway, demonstrated cell death independent of Notch – uncovering the proteasome as its main target. The effects of GSIs recently developed and/or used in clinical trials are currently being studied to understand their action on breast cancer cell death. Similarly, proteasome inhibitors are a recent line of anticancer drugs that have been shown to cause cancer cell death and are still currently being investigated [5].

Using several breast cancer cell lines, Han and colleagues demonstrated that common GSIs such as DAPT and L-685,458 inhibited γ-secretase and intracellular Notch

ER = estrogen receptor; GSI = γ-secretase inhibitor.

domain formation, but had no effect on cell viability and death. With Z-LeuLeuNleu-CHO (GSI-I), however, they were able to induce cell death via proteasomal inhibition and reduce γ-secretase activity. Interestingly, they observed no apparent effects of DAPT or L-685,458 on the proteasome. The authors therefore concluded that Z-LeuLeuNleu-CHO promoted cell death predominantly through proteasome inhibition. In contrast, a study by Farnie and colleagues found that DAPT reduced mammosphere formation by 22% compared with vehicle [6]. In accordance, Meurette and colleagues demonstrated that DAPT reduced Akt phosphorylation in MCF-7 and DCIS cell lines and increased sensitivity to the chemotherapeutic class of nitrogen mustard alkylating agents, known as melphalan (Alkeran) [7]. These studies indicate that DAPT inhibited tumor-initiating breast cancer cell growth and increased sensitivity to chemotherapeutic agents. The importance of inhibiting the Notch pathway might therefore be context dependent.

The authors do comment on the discrepancies of their findings compared with previous published literature on the basis of employing different experimental strategies. GSI-I induced G_2/M arrest and apoptosis in breast cancer cell lines with effects on both γ-secretase activity and the proteasome [8]. In agreement with Han and colleagues' results, similar studies indicated that GSI-I inhibits HER2-overexpressing breast tumor-initiating sphere formation, which was not apparent in MCF-7 cells that express low to moderate levels of HER2 [9]. Interestingly, Han and colleagues showed that proteasome inhibition using lactacystin reduced cell viability in ERα$^-$SKBr3 cells and MDA-MB-231 cells, with little effect on ERα$^+$MCF-7 cells. Could this observation suggest that the level of proteasome activity differs between breast cancer subtypes and that the degree to which GSI-I functions as a proteasome inhibitor would depend on high proteasome activity? With this in mind, their novel findings unfurled a potential mechanism that could be advantageous.

Han and colleagues' results could suggest a potential duality in therapeutic treatment of breast cancer cells. Proteasome inhibition could be a vital target of treatment in combination with other oncogenic or growth-promoting proteins. Evidence suggests the importance of inhibiting the Notch pathway in combination with current targeting of ERα [10] or HER2 [11]. For example, recent studies have shown that GSI-I or Ly 411,575 treatment of triple-negative MDA-MB-231 cells or treatment in combination with tamoxifen in ERα$^+$T47D:A18 cells arrested growth and caused tumor regression *in vivo*, respectively [10]. In the HER2$^+$ breast cancer cell line, BT474, Ly 411,575 or MRK-003 GSI treatment increased apoptosis and re-sensitized resistant HER2$^+$ cells to trastuzumab [12]. Furthermore, these two studies showed that specific knockdown of Notch-1 by siRNA inhibited cell proliferation and increased sensitivity to either 4-hydroxytamoxifen or trastuzumab. These articles demonstrated the importance of Notch-1 signaling in ERα$^-$, ERα$^+$, or HER2$^+$ breast cancer cells [10,12]. GSIs are therefore currently in clinical trials as anticancer drugs, but specific pharmacologic and molecular properties are still being investigated.

Because little is known about which GSIs will be the most advantageous in the clinic, it is important to understand structural and biochemical differences between specific compounds. Han and colleagues used the Z-LeuLeuNleu-CHO GSI, which is a derivative of the proteasome inhibitor MG-132. The structure of MG-132 is Z-LeuLeuLeu-CHO. GSI-I is very close structurally, differing only by a single norleucine substitution: Z-LeuLeuNorLeu-CHO. Treatment with two structurally similar drugs targeted at different enzymes – that is, γ-secretase or the proteasome – could therefore be anticipated to inherently have shared effects, which could account for the results on the proteasome seen by GSI-I.

Furthermore, current GSIs that are being used in clinical trials are a new line of medicinally and structurally modified chemical molecules that could show different effects. DAPT and L-685,458 GSIs, which were used in Han and colleagues' article, are not used in current trials and are structurally unique. Structurally modified GSIs are still being investigated and could provide evidence for cellular death via inhibition of the Notch pathway independent of the proteasome. Understanding these key properties may lead to better therapeutic targeting. Studying the effect of these new classes of GSIs in clinical trials on proteasome activity could prove very interesting.

In conclusion, in the present article we comment that Z-LeuLeuLeu-CHO is a derivative of a proteasomal inhibitor, which could inherently have shared effects; that current GSIs used in clinical trials are structurally unique and their effects on the proteasome are as yet unknown; and that the level of proteasomal activity, which could differ between breast cancer subtypes, may play a critical role by which GSI-I functions to inhibit growth. In summary, Han and colleagues beg the intriguing question of whether proteasome inhibitors independently or in combination with GSIs or with current chemotherapeutic agents provide novel and exciting therapeutic strategies against breast cancer.

Competing interests

The authors declare that they have no competing interests.

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