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Gamma-secretase inhibitors

Do they have a role in the treatment of B cell lymphoma?

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Gamma secretase inhibitors (GSIs) make up a new class of drugs targeting the Notch signal transduction pathway that are currently in clinical trials for a variety of malignancies and Alzheimer's disease. The target of GSIs, γ-secretase, is a large multi-protein transmembrane complex composed of presenilin, Aph1, Pen-2 and nicastrin (See **Fig. 1**).[1] The role of γ-secretase in a variety of physiological and pathological processes is derived from its activity as a protease against Notch, a central molecule in the control of growth and differentiation.[2] γ-secretase also cleaves amyloid precursor protein, contributing to the generation of the peptide fragments that comprise the amyloid plaques seen in the Alzheimer's disease.[3] Notch is a transmembrane protein with an extracellular domain that acts as a receptor for the DSL (Delta, Serrate, lag-2) family of ligands.[4] Engagement of the extracellular Notch receptor by its cognate ligand triggers cleavage of Notch in the extracellular domain by α-secretase, followed by γ-secretase cleavage of the hydrophobic transmembrane domain. This process generates the intracellular fragment of Notch (Notch IC), which is the active agent in modulating transcriptional gene regulation by the Notch pathway.

Notch signal transduction is an essential aspect of gene regulation during differentiation and development. Once activated, Notch IC translocates to the nucleus and binds to the DNA binding protein CBF1 (RBJk, CSL). CBF1 in its basal state is complexed to co-repressor proteins that inhibit transcription from CBF1-bound promoters. Notch IC binding to CBF1 causes displacement of co-repressors and recruitment of co-activators, especially members of the MAML (mastermind-like) family, and activation of a large number of target genes.[5] Several of these target genes are themselves transcriptional repressors, such as Hey1/Hes1, resulting in complex changes in the transcriptional profile of the cell in which Notch activation occurs (reviewed in ref. 6). To further add to the complexity of the system, there are four Notch family members that are expressed in different patterns that are cell type-dependent. Thus the specific effects of Notch activation (or inhibition) in a particular tumor are not predictable a priori. However, Notch activation plays an important role in the genesis of T cell acute lymphoblastic leukemia (T-ALL). Activating mutations of Notch are found in >50% of T-ALL, and translocations of Notch that result in a truncated, constitutively active Notch occur.[6] In contrast, the role of Notch activation in B cell lymphoma and leukemia is not clear. Notch activity in several Burkitt lymphoma cell lines was found to be low and the Notch target HES was not activated.[7] However, follicular dendritic cells bearing Notch ligands activate Notch and protect germinal center B cells from apoptosis.[8] There are several reports of Notch activation in Hodgkin's lymphoma, multiple myeloma and chronic B cell lymphocytic leukemia (B-CLL) and growth inhibition upon treatment with GSIs.[9-13] In contrast, however, constitutively active Notch was reported to cause the growth arrest and apoptosis of cells from a wide range of human B cell malignancies as did Hes1

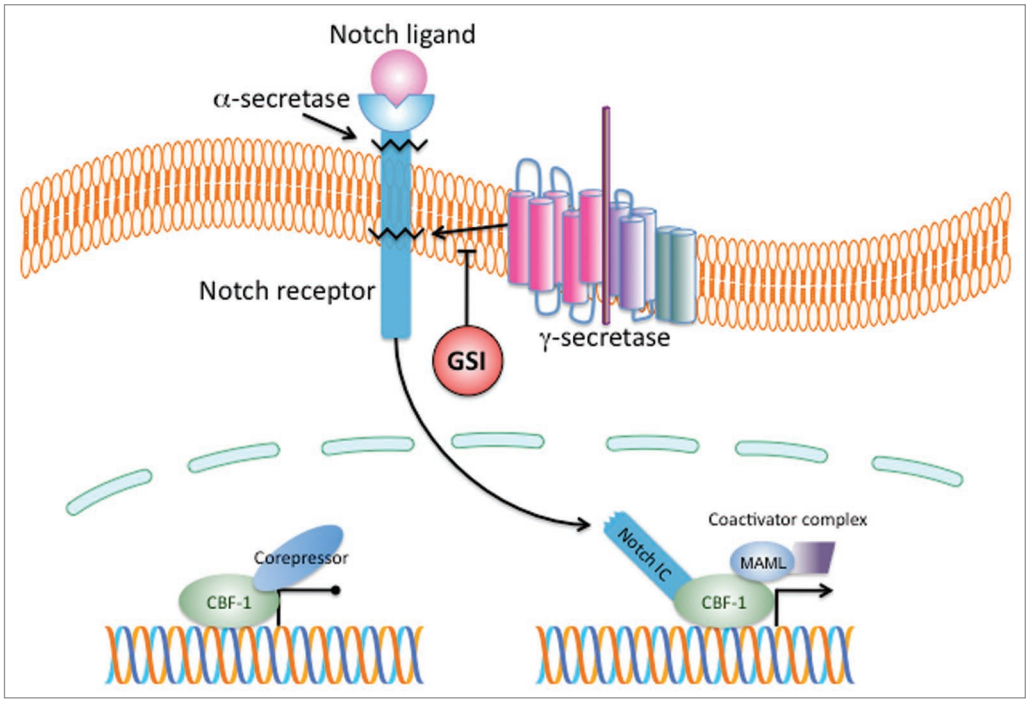

Figure 1. A Notch ligand (which may be present on the surface of other cells) is shown occupying the extracellular portion of the Notch receptor molecule. Binding leads to cleavage of Notch by α-secretase and by γ-secretase at two separate sites, releasing the intracellular domain of Notch (Notch IC). The transmembrane γ-secretase complex is shown as a complex of four molecules (the number of transmembrane domains has been reduced for ease of representation). Notch IC translocates to the nucleus and binds to the cellular transcription regulator protein CBF1 (CSL, RB-Jk), leading to dissociation from co-repressor molecules, recruitment of co-activator proteins, particularly MAML and histone acetyltransferases, and activation of gene transcription at numerous Notch-responsive genes. γ-secretase inhibitors (GSI) inhibit the enzymatic activity of γ-secretase and prevent Notch activation.

when overexpressed by retroviral transduction.[14] Thus the effect of GSI treatment of B cell malignancies may not be predictable and might even enhance growth or survival of malignant cells under certain conditions.

It is in this setting that Lan and colleagues investigated the effect of γ-secretase inhibition in KSHV-associated primary effusion lymphoma (PEL).[15] PEL is a rare form of B cell lymphoma associated with infection by the human gamma herpesvirus Kaposi's sarcoma-associated virus (KSHV).[16] PEL presents in immunosuppressed patients as malignant effusions in body cavities such as the peritoneum. PEL cells have a transcriptional profile that resembles plasma cell tumors most closely, regardless of the status of Ig gene rearrangement or EBV-infection, which are also present in the majority of PELs.[17] Lan et al. had previously shown that KSHV latency-associated protein (LANA), which is required for nuclear KSHV episome maintenance and has multiple other gene regulatory functions, binds and antagonizes the function of cellular Sel110, an E3 ligase which destabilizes Notch IC.[18] LANA therefore significantly extends the half-life of Notch IC in cells latently infected by KSHV. In this report, they demonstrate that GSIs inhibit PEL tumor growth in a SCID mouse model, extending previous findings that GSIs inhibit the growth of PEL cells in vitro.[19] Mice were injected intraperitoneally with PEL cells or KSHV-negative Burkitt lymphoma (BL) cells and a cell-permeable dipeptide GSI (DAPT) was administered daily to treatment groups beginning 5 d after tumor injection. GSI-treated mice lived 25–40% longer than mock-treated mice, although both groups eventually succumbed after 50–70 d. Importantly, KSHV-negative BL cells, which did not display constitutive Notch activation, were not affected by GSI treatment, indicating specificity of response. The tumors in the GSI-treated mice underwent extensive necrosis, which had not been previously observed in vitro. Caspase 3 staining of the tumors indicated that GSIs also induced some degree of apoptotic cell death. It should be noted that one of the cell lines used in this study came from an EBV-negative PEL and the other from an EBV-positive PEL. The EBV status and pattern of EBV gene expression is particularly relevant in analyzing GSI effects, as EBNA 2, one of the EBV proteins expressed during latent infection, co-opts the Notch signaling pathway.[20-22] EBNA2 binds to CBF1/RBJk, and relieves repression, similar to Notch IC, thereby constitutively activating the pathway. Although EBNA2 is usually not expressed at high levels in BL or PEL cells, the potential exists for EBNA2 to counteract the effects of GSI treatment.

Despite promising preclinical studies such as the current one, there are several significant hurdles to establishing GSIs as a standard component of cancer chemotherapy regimens. GSIs have been shown to inhibit growth and induce apoptosis in xenograft models for a variety of solid tumors and hematologic malignancies, including Kaposi's sarcoma, breast cancer,

pancreatic cancer, multiple myeloma and T cell lymphoma, and they are currently in clinical trials for several malignancies.[23] However, significant dose-limiting gastrointestinal toxicity characterized by goblet cell metaplasia has been observed as a side effect of many GSIs. Further, even in human T-ALL treatment trials, where a clear relationship between Notch mutations and malignant phenotype exists, only a limited anti-leukemic effect of GSIs has been attained. Another caveat has recently been raised by the finding that the anti-breast cancer effects of some GSIs may actually be due to inhibition of the proteasome rather than Notch-IC.[24] In addition to these challenges of drug toxicity and off target effects, the sheer complexity of Notch signaling crosstalk and cell-specific differences will likely require individualization of treatment regimens.

A recent study of GSIs in T-ALL suggests how some of these problems might be overcome.[25] There seems to be a reciprocal relationship between Notch-IC inhibition and glucocorticoid resistance that affects both gut toxicity and apoptosis of T-ALL cells. Inhibition of Notch-IC allowed increased transcription of the glucocorticoid receptor gene in T-ALL cells, resulting in restored steroid sensitivity. Conversely, GSI treatment in combination with dexamethasone led to greater expression of glucocorticoid-responsive genes that suppressed the gastrointestinal toxicity of GSIs. Thus, treatment of mice with both glucocorticoids and GSIs resulted in decreased toxicity as well as increased anti-leukemic effect. Such combinations of pathway-specific drugs may be required to achieve maximal therapeutic benefit from GSIs. It is clear that many hurdles remain before the potential of GSIs in treatment of B cell lymphoma is realized. However, it is not unrealistic to envision a day in the near future when biopsies are analyzed at the molecular level to assess Notch activation prior to formulation of a patient-specific multimodality regimen. The current study by Lan et al. points out one way to potentially improve the treatment options for patients with KSHV-associated PEL, which has a dismal prognosis. SS was supported by grants CA 81133 and CA 119905 from the NIH/NCI.

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