EDITORIAL

MDR-1, Bcl-xL, *H. pylori*, and Wnt/ β -catenin signalling in the adult stomach: how much is too much?

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Multiple drug resistance (MDR) is a major cause of failure of chemotherapy in cancer treatment. The membrane transporter P-glycoprotein (MDR-1, Pgp) encoded by the adenosine triphosphate-binding cassette, subfamily B, member 1 is the main mechanism for decreased intracellular drug accumulation in MDR cancer.¹ Increases in Mdr-1 expression prevent tumor cells from a variety of induced apoptosis, but how this occurs is poorly understood. It is essential to understand how this occurs to be able to design effective therapeutic interventions. The study by Rocco *et al*² (this issue) clearly shows that in the mitochondria of gastric cancer cell lines MDR1 physically interacts with Bcl-xL, a well-defined antiapoptotic effector. Furthermore, this elegant study compares MDR-1 expression in the gastric mucosa of spontaneously aborted human fetuses compared with normal adult mucosa, and as well reports on the presence of MDR-1 in *Helicobacter pylori* (HP) negative, positive chronic gastritis and those with intestinal metaplasia and intestinal type gastritis. Together their interesting findings support the idea that in the stomach, MDR-1 behaves as an oncofetal protein, which during HP-related gastric carcinogenesis may cross-talk with Bcl-xL to work in an antiapoptotic manner.

The critical findings of Rocco *et al*² are illustrated in Figure 1a. MDR-1 is increased and is localized to the mitochondria and presumably the plasma membrane of AGS and MKN-28 cell lines. These lines each contain well-defined mutations and characterized responses to Akt and MAPK inhibitors.³ Importantly, there is physical

interaction between the antiapoptotic protein Bcl-xL and MDR-1. Knockdown of MDR-1 increases the apoptotic index of these cells exposed to oxidative stress consistent with a role for MDR-1 in apoptosis. Several questions emerge from these findings. The first is why is MDR-1 increased in some HP-positive mucosa but in 100% of the intestinal metaplasia samples? A likely causative effector of the increase in MDR-1 is the activation of canonical or Wnt/ β -catenin signaling. It has been known for a dozen years that the MDR-1 gene may be stimulated by Tcf4.⁴ Yamada *et al*⁴ clearly demonstrated the presence of Tcf4 sites on the MDR-1 promoter and showed that MDR-1 protein had substantially increased in adenomas and colon cancer.

Wnts are palmitolated glycoproteins that have key effects in development, inflammation, stem cell maintenance, and cancer.^{5,6} There are two types of Wnt signaling. Canonical or Wnt/ β -catenin signaling regulates the concentration of the effector β -catenin. Noncanonical Wnt signaling in general will inhibit β -catenin signaling and/or stimulate JNK, PKC and $[Ca^{2+}]_i$ release.⁷ In the absence of Wnt signaling, the amounts of the effector β -catenin are tightly regulated by a complex of scaffold proteins, Auxin and the tumor suppressor adenomatous polyposis coli (APC) together with GSK-3 β . When a Wnt protein binds its two co-receptors Frizzled (Fzd) and lipoprotein-related peptide 6 (LRP6), the auxin-APC scaffold releases β -catenin. The Wnt-stimulated Fzd recruits dishevelled (Dvl) to the trimeric Wnt-Fzd-LRP6 receptor complex. This activated Dvl then interacts with auxin and GSK-3 β to form a LRP6-associated ‘signalsome’

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where GSK-3 β phosphorylation of β -catenin is prevented. Recent experiments demonstrate that Wnt induces the association of the intact scaffold complex, even when APC is truncated, with phosphorylated LRP6.⁸ The released β -catenin migrates to the nucleus to stimulate Lef/Tcf transcription factors to activate a large number of Wnt target genes such as C-Myc, CyclinD1, and Sox9. How GSK-3 β activity is inhibited by Wnt stimulation has been extensively studied and requires sequestration in multi-vesicular bodies.⁹ In sporadic colorectal cancers mutations in APC are found in ~80% of the cancers. While most of the steps leading to colorectal cancer have been determined,⁵ knowledge of the same sequence of APC and β -catenin mutations in stomach cancer is just beginning to be understood.

Strong evidence that altered Wnt/ β -catenin signaling in the stomach epithelia rapidly generates an initiating step in gastric tumorigenesis has come from recent studies deleting APC or GSK-3 β , as well as expression of a constitutively active β -catenin protein in inducible mouse models.¹⁰ This elegant study found that activating Wnt/ β -catenin signaling (using floxed truncated APC, floxed inactivated GSK-3 β , or constitutively activated β -catenin mice) after Cre induction, generated in the antral glands a rapid development of small hyperproliferative microradenomas. With age, these small lesions developed into large adenomas, and IHH demonstrated high levels of c-MYC and Sox9. In the corpus region, the same

Cre induction generated a progressive loss of parietal cells with fundic gland polyposis and adenomas comparable with those observed in the antrum.

Thus in both the antrum and corpus, activation of Wnt/ β -catenin signaling alone was not sufficient to drive malignancy. We know from the elegant work of the Oshima group that Wnt1 (which is upregulated in later stage gastric cancer)¹¹ when present together with PGE₂ will generate an invasive gastric adenocarcinoma formation by 1 year.¹² Consistent with this work, the CK19 Cre-Wnt1 mice show higher levels of nuclear β -catenin accumulation and pre-neoplastic lesions but not tumorigenesis. So, stimulation of Wnt/ β -catenin signaling could lead to microadenomas in the antrum and hyperproliferation in the corpus with increased expression of MDR-1 (Figure 1b). The cell type responsible for Wnt1 secretion in the stomach is not known in detail but IHH has shown Wnt5a at the base of the normal gastric corpus mucosa.¹³

However, later stages of gastric cancer (intestinal type and diffuse type) correlate with an increase in Wnt5a protein expression. In gastric cancer, Wnt5a has been shown to activate the small GTP-binding protein Rac1, which has an established role in migration and metastasis.¹⁴ Recent reports document that polyclonal antibodies against Wnt5a inhibit invasion and metastasis of a variety of gastric cancer cell lines.¹⁵ While the gastric cancer cell lines secrete Wnt5a to

Figure 1 (a) Multiple drug resistance (MDR)-1 physically interacts with the antiapoptotic Bcl-2 family member Bcl-xL on the mitochondria of gastric cancer cell lines and is present on mucosa of fetal stomach, then lost, but increased with intestinal type gastritis. (b) Increased Wnt/ β -catenin signaling, initiated by either Wnt1 or Wnt5a, could increase MDR-1.⁴ The degradation complex composed of Axin1 and adenomatous polyposis coli is linked to the Wnt/Fzd (frizzled)/LRP6 while β -catenin is released.⁸ *H. pylori* can stimulate LRP6 phosphorylation, and TNF α from macrophages can increase Wnt/ β -catenin signaling¹⁹ in mouse stomach. Normal stomach turnover requires some Wnt/ β -catenin signaling,^{24,25} but how much is too much?

various degrees, the source of Wnt5a in the late stage of gastric cancer appears to be epithelial; both intestinal type and diffuse-adherent and -scattered demonstrated increased Wnt5a by IHH.¹³ The determinants of Wnt5a secretion from these types of cells are unknown. However, work with colon adenocarcinoma cells has shown a truncated APC was necessary for Wnt5a secretion.¹⁶

The *H. pylori* samples in the Rocco study² showed some expression of MDR-1 while the intestinal metaplasia samples showed 100%. Is it likely that a threshold limit of Wnt/ β -catenin signaling is has been surpassed in the latter, while the canonical Wnt signaling activated by *H. pylori*¹⁷ is insufficient to increase MDR-1? *H. pylori* is well known to stimulate LRP6 phosphorylation, a gold-standard for Wnt/ β -catenin signaling.¹⁸ Indeed there is evidence that CagA can also disperse β -catenin from E-cadherin- β -catenin complexes to further increase the concentration of free β -catenin capable of migrating to the nucleus. Other *H. pylori* constituents such as VacA, OlpA, and peptidoglycan can activate PI3K, which will stimulate Akt to then cause ser9 phosphorylation of GSK-3 β , inhibiting GSK3 β activity.¹⁷ Another major player in this story with *H. pylori* infection is TNF α from infiltrating macrophages.¹⁹ The TNF α promoted Wnt/ β -catenin signaling in K19Wnt1 mouse stomach, and macrophage deletion in APC Δ^{716} mice suppressed intestinal tumorigenesis. Indeed, the importance of increased TNF α was clearly demonstrated in a colitis-associated colon cancer model where TNFR1 signaling in bone marrow-derived cells enhanced Wnt/ β -catenin signaling in the epithelia, and TNFR1 knockout mice did not generate the same tumor number or load.²⁰ Thus *H. pylori* will stimulate some proximal Wnt/ β -catenin signaling while recruiting macrophage infiltration, which will further activate more Wnt/ β -catenin signaling (Figure 1b).

MDR-1 is physically associated with Bcl-xL in mitochondria in the current study (Figure 1a). Most conventional chemotherapeutic agents exert their cytotoxic effect by inducing apoptosis via the intrinsic or mitochondrial pathway.²¹ Survival is increased by expression of antiapoptotic proteins. Bcl-xL is an antiapoptotic member of the Bcl-2 family. Bcl-xL prevents apoptosis by inhibiting release of DIABLO and cytochrome c into the cytoplasm. In general, Bcl-xL will sequester BAX, BAD, and other 'activator' proteins, which increase mitochondrial membrane permeability.

The design of small molecules that block Bcl-xL interaction with these and other proteins is a rapidly growing endeavor²² in order to overcome resistance to conventional chemotherapy. Analyses of mitochondria to determine the relative amounts BH3 proteins or antiapoptotic multidomain homologs (such as Bcl-xL) are present can guide therapy. An example of such mitochondrial profiling, which predicted the activity of Bcl-2 antagonists allowing a more designed therapy has been demonstrated in neuroblastoma.²³ Is it possible to intervene earlier in the development of gastric cancer?

If the continuum of gastric cancer is initiated with altered Wnt/ β -catenin signaling leading to increased MDR-1 expression (Figure 1b), then would inhibit either Wnt secretion or events distal to Wnt signalsome formation attenuate Wnt/ β -catenin signaling to a level, which prevents MDR-1 increases but still allows some Wnt/ β -catenin signaling to occur? Some Wnt/ β -catenin signaling is required for normal stomach turnover—there are Lgr5 + stem cells present in mouse pylorus. When Lgr5 + cells are isolated they will grow expanding gastric organoids. This growth requires exogenous Wnt3a. For differentiated lineages to appear, Wnt3a must be removed.²⁴ No human gastric organoid cultures have been reported.²⁵ The efficacy of small molecule Wnt pathway modifiers have recently been reviewed.^{5,26} Indeed the recent structural information of a Wnt8-Fzd8-CRD complex will enable rational drug designs to alter Wnt-receptor interactions.²⁷ While there is some specificity in these pathway modifiers, new targets are also being studied. One potential target is particularly interesting. Wntless (or Evi/Gpr177) is a cargo receptor, which carries Wnt protein from the Golgi to the plasma membrane. It is required for exocytosis of Wnt proteins. Wntless is a highly conserved seven-pass transmembrane protein. Notably, Wntless is overexpressed in human astrocytic gliomas relative to normal brain. Loss of Wntless stopped glioma growth *in vivo* and *ex vivo*.²⁸ Does an increase in Wntless/Evi/Gpr177 precede or allow the increased Wnt1 and Wnt5a secretion to give rise to increased Wnt/ β -catenin signaling (which then increases MDR-1)? If Wntless is increased at one stage of stomach cancer development, could a Wntless inhibitor prevent the subsequent increases in MDR-1?

In conclusion, the study by Rocco *et al*² provides a focal point to imagine how Wnt/ β -catenin signaling can be manipulated to attenuate

MDR-1 expression to prevent multiple drug resistance.

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DISCLOSURE/CONFLICT OF INTEREST

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