Therapeutically targeting glypican-3 via a conformation-specific single-domain antibody in hepatocellular carcinoma

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AUTHOR SUMMARY

Liver cancer is the fifth most prevalent neoplasm in the world and is the third most common cause of cancer-related death (1). Most liver cancer cases affect the cells of the liver and therefore are known as “hepatocellular carcinoma.” The glypican-3 (GPC3) protein, which plays a role in cell proliferation, has emerged recently as a candidate therapeutic target because it is highly expressed in hepatocellular carcinoma but not in normal adult liver tissue. Here, we report a human monoclonal antibody, HN3, with high affinity for GPC3 molecules on the cancer cell surface. We found that HN3 inhibited proliferation of hepatocellular carcinoma cells and exhibited tumor growth inhibition in mice, suggesting a potential role in GPC3-targeted cancer therapy.

The biological functions of GPC3 and its role in liver tumorigenesis remain elusive. A previous study showed that hepatocarcinoma cells infected with lentivirus expressing soluble GPC3, a secreted form of the protein lacking the glycosylphosphatidylinositol (GPI) domain that normally anchors the protein to the cell membrane, did not multiply as quickly as control cells (2). Another study showed that a recombinant soluble GPC3 inhibited the growth of hepatocarcinoma in vitro, possibly by competing with endogenous GPC3 for binding to growth factors on the cancer cell surface (3). These findings suggest that GPC3 plays a role in the proliferation of hepatocarcinoma cells. To date, several mouse monoclonal antibodies against GPC3 have been produced (4), and one, GC33, is under evaluation in a phase I clinical trial (5); however, almost all of these antibodies recognize a peptide derived from GPC3. None of the antibodies has been reported to inhibit cell proliferation directly or to kill cells, possibly reflecting the difficulty of neutralizing the proliferative function of GPC3 by a conventional antibody. Domain antibodies are small in size and therefore have the potential to target functional sites on the surface of an antigen (e.g., the clefts of enzymes and receptors).

In the present study, we isolated HN3, a single-domain antibody targeting GPC3, by phage display technology. Phage display is a laboratory technique for the high-throughput screening of antibodies displayed on bacterial viruses (known as “phage”). Cell binding is one of the most important features in the design of therapeutic antibodies capable of targeting cell-surface proteins. Our analysis showed that HN3 bound GPC3-expressing hepatocellular carcinoma tumor cells with high affinity and specificity (Fig. PL4). Because HN3 has a human origin and displays high affinity, it is expected to be less immunogenic than murine antibodies and to be efficient in targeting GPC3-expressing tumors. We characterized the binding properties of HN3 and found that it recognized a site in the native, or naturally occurring, form of GPC3 on cancer cells. In particular, the binding of HN3 required both the N- and C-terminal domains of GPC3. This unique feature distinguishes HN3 from all the known monoclonal antibodies that recognize either the N or C termini of GPC3. To investigate the antitumor activity of HN3, we tested the antibody in our hepatocarcinoma cell model and found that HN3 inhibited the proliferation of GPC3-positive hepatocellular carcinoma cells in vitro (Fig. PI4B). To evaluate the therapeutic potential of the HN3 antibody in liver cancer, we examined the binding of the antibody on hepatocellular carcinoma tumor tissues and tested the antitumor activity of HN3 in mice. We found that HN3 specifically stained hepatocellular carcinoma tissues from patients (Fig. PlC and D). Furthermore, the antibody significantly inhibited the growth of hepatocellular carcinoma tumors in nude mice, an indication of therapeutic potential.
immunodeficient animal model (Fig. P1 E and F). Finally, to understand the underlying mechanism of HN3 action, we analyzed cell-signaling pathways in hepatocellular carcinoma cells treated with HN3 and found that HN3 induced cell-cycle arrest at G1 phase through Yes-associated protein (yap) signaling. This observation could help uncover the role of GPC3 in Hippo–yap signaling in liver cancer progression.

Here, we describe a human monoclonal antibody that targets GPC3. The unique feature of the HN3-binding site may reveal a previously undescribed function of GPC3, because HN3 shows unprecedented and direct inhibition of GPC3-positive cell proliferation. Furthermore, HN3 inhibits liver tumor growth in mice and should be evaluated further as a therapeutic candidate for the treatment of liver cancer.