Therapeutic targeting of the thrombospondin-1 receptor CD47 to treat liver cancer

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Received: 30 January 2015 / Accepted: 30 January 2015 / Published online: 18 March 2015
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Abstract CD47 is a signaling receptor for the matricellular protein thrombospondin-1 and a counter-receptor for signal regulatory protein- α (SIRP α) on macrophages. Following its initial discovery in 1992 as a cell surface protein that is over-expressed by ovarian carcinoma, elevated CD47 expression has emerged as a negative prognostic factor for a variety of cancers. CD47 is also a potential therapeutic target based on the ability of CD47 blockade to cause regression of tumors in mice, and a humanized CD47 antibody has recently entered phase I clinical trials. CD47 blockade may control tumor growth by inhibiting thrombospondin-1 signaling or by preventing inhibitory SIRP α signaling in tumor-associated macrophages. A recent publication by Lee et al. (Hepatology 60:179–191, 2014) provides evidence that blocking CD47 signaling specifically depletes tumor-initiating stem cells in hepatocellular carcinoma and implicates cathepsin-S/protease-activated receptor-2 signaling in mediating this therapeutic response.

Keywords CD47 · Tumor-initiating cells · Cathepsin-S · NF κ B · Chemoresistance

Studies of the mechanisms underlying acquired resistance to conventional chemotherapy have been advanced by emergence of the cancer stem cell hypothesis (O'Connor et al.

2014). In most cancers, only a minor subpopulation of cells has the ability to form a new tumor when transplanted into healthy animals. These tumor-initiating cells (TICs) bear characteristic stem cell markers and have the property of self-renewal. TICs are more resistant to conventional chemotherapy, leading to rapid regrowth of tumors and, ultimately, treatment failure. Thus, new therapeutic strategies are needed to selectively kill TICs.

CD47 is a cell surface protein that exhibits elevated expression on a growing list of cancer types (Campbell et al. 1992; Chao et al. 2012; Willingham et al. 2012). Consistent with high CD47 expression being a negative prognostic factor in some cancers, CD47 is expressed highly on TICs from these cancers. Treatment of tumor-bearing mice with antibodies that block CD47 signaling has produced impressive tumor regression when used alone or combined with existing therapeutics (Zhao et al. 2011; Chao et al. 2012; Willingham et al. 2012), and humanized CD47 antibodies have recently entered human clinical trials (NCT02216409, NCT02367196). However, the mechanism by which these antibodies act remains controversial (Soto-Pantoja et al. 2012; Zhao et al. 2012). One theory proposes that the antibodies prevent CD47 on the tumor cells from engaging SIRP α on tumor-associated macrophages (Chao et al. 2012). The inhibitory SIRP α signaling triggered by CD47 binding is well documented to inhibit the phagocytic activity of macrophages (Barclay and van den Berg 2014). In this model, CD47 serves as a “don’t eat me” signal on the tumor cells. On the other hand, SIRP α -independent models invoke CD47 signaling that cell-autonomously regulates tumor cell viability, resistance to therapy, or the ability of antibody or cytotoxic T cell immunity to eliminate tumor cells (Zhao et al. 2011; Boukhari et al. 2015; Soto-Pantoja et al. 2014; Lee et al. 2014).

Based on their finding that CD47 is enriched on CD133⁺/CD24⁺ TICs isolated from a hepatocellular carcinoma cell line and is elevated by serial passage in the presence of

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doxorubicin and cisplatin, Lee and colleagues examined cell-autonomous mechanisms by which high CD47 expression confers chemoresistance and increases the stem cell character of TICs (Lee et al. 2014). Microarray data identified elevated cathepsin-S mRNA in the TICs that was decreased when the cells differentiated and when CD47 expression was suppressed using a specific shRNA. CD47 expression correlated with that of cathepsin-S in hepatocellular carcinoma patient specimens and was shown to regulate cathepsin-S expression in TIC through activation of NF κ B. Cathepsin-S in turn controlled TIC migration and invasion through activation of protease-activated receptor-2, which is a substrate for this protease. Finally, the authors demonstrated that antisense morpholino suppression of CD47 suppressed the growth and metastasis of hepatocellular carcinoma xenografts in nonobese diabetic (NOD)/SCID mice. Combination with doxorubicin treatment enhanced the activity of the morpholino. Although the latter experiments are consistent with the authors' proposed mechanism, the variant SIRP α expressed in NOD/SCID mice can recognize the human CD47 expressed by the hepatocellular carcinoma cells (Takenaka et al. 2007). Thus, loss of the "don't eat me" signal may also contribute to the observed therapeutic response.

These interesting results are consistent with another new publication that links CD47 signaling in glioblastoma cells to NF κ B activation and the cyclin-dependent kinase inhibitor p16^{INK4A} (Boukhari et al. 2015). In contrast to the cell-autonomous beneficial effects of high CD47 expression in TICs, thrombospondin-1 signaling through CD47 limits self-renewal and suppresses expression of the stem cell transcription factors cMyc, Sox2, Oct4, and Klf4 in nontransformed cells (Kaur et al. 2013). Further study is required to understand why CD47 differentially regulates cancer versus normal stem cells and to understand the role of the CD47 ligand thrombospondin-1, which is often silenced during malignant progression (Isenberg et al. 2009), in TIC maintenance.

Acknowledgments This work was supported by the Intramural Research Program of the National Institutes of Health, National Cancer Institute, Center for Cancer Research.

References

- Barclay AN, van den Berg TK (2014) The interaction between signal regulatory protein alpha (SIRP α) and CD47: structure, function, and therapeutic target. *Annu Rev Immunol* 32:25–50
- Boukhari A, Alhosin M, Bronner C, Sagini K, Truchot C, Sick E, Schini-Kerth VB, Andre P, Mely Y, Mousli M, Gies JP (2015) CD47 activation-induced UHRF1 over-expression is associated with silencing of tumor suppressor gene p16INK4A in glioblastoma cells. *Anticancer Res* 35:149–157
- Campbell IG, Freemont PS, Foulkes W, Trowsdale J (1992) An ovarian tumor marker with homology to vaccinia virus contains an IgV-like region and multiple transmembrane domains. *Cancer Res* 52:5416–5420
- Chao MP, Weissman IL, Majeti R (2012) The CD47-SIRP α pathway in cancer immune evasion and potential therapeutic implications. *Curr Opin Immunol* 24:225–232
- Isenberg JS, Martin-Manso G, Maxhimer JB, Roberts DD (2009) Regulation of nitric oxide signalling by thrombospondin 1: implications for anti-angiogenic therapies. *Nat Rev Cancer* 9:182–194
- Kaur S, Soto-Pantoja DR, Stein EV, Liu C, Elkhouloun AG, Pendrak ML, Nicolae A, Singh SP, Nie Z, Levens D, Isenberg JS, Roberts DD (2013) Thrombospondin-1 signaling through CD47 inhibits self-renewal by regulating c-Myc and other stem cell transcription factors. *Sci Rep* 3:1673
- Lee TK, Cheung VC, Lu P, Lau EY, Ma S, Tang KH, Tong M, Lo J, Ng IO (2014) Blockade of CD47-mediated cathepsin S/protease-activated receptor 2 signaling provides a therapeutic target for hepatocellular carcinoma. *Hepatology* 60:179–191
- O'Connor ML, Xiang D, Shigdar S, Macdonald J, Li Y, Wang T, Pu C, Wang Z, Qiao L, Duan W (2014) Cancer stem cells: a contentious hypothesis now moving forward. *Cancer Lett* 344:180–187
- Soto-Pantoja DR, Miller TW, Frazier WA, Roberts DD (2012) Inhibitory signaling through signal regulatory protein- α is not sufficient to explain the antitumor activities of CD47 antibodies. *Proc Natl Acad Sci U S A* 109:E2842
- Soto-Pantoja DR, Terabe M, Ghosh A, Ridnour LA, Degraff WG, Wink DA, Berzofsky JA, Roberts DD (2014) CD47 in the tumor micro-environment limits cooperation between antitumor T-cell immunity and radiotherapy. *Cancer Res* 74:6771–6783
- Takenaka K, Prasolava TK, Wang JC, Mortin-Toth SM, Khalouei S, Gan OI, Dick JE, Danska JS (2007) Polymorphism in Sirpa modulates engraftment of human hematopoietic stem cells. *Nat Immunol* 8:1313–1323
- Willingham SB, Volkmer JP, Gentles AJ, Sahoo D, Dalerba P, Mitra SS, Wang J, Contreras-Trujillo H, Martin R, Cohen JD, Lovelace P, Scheeren FA, Chao MP, Weiskopf K, Tang C, Volkmer AK, Naik TJ, Storm TA, Mosley AR, Edris B, Schmid SM, Sun CK, Chua MS, Murillo O, Rajendran P, Cha AC, Chin RK, Kim D, Adorno M, Raveh T, Tseng D, Jaiswal S, Enger PO, Steinberg GK, Li G, So SK, Majeti R, Harsh GR, van de Rijn M, Teng NN, Sunwoo JB, Alizadeh AA, Clarke MF, Weissman IL (2012) The CD47-signal regulatory protein alpha (SIRP α) interaction is a therapeutic target for human solid tumors. *Proc Natl Acad Sci U S A* 109:6662–6667
- Zhao XW, van Beek EM, Schornagel K, Van Der Maaden H, Van Houdt M, Otten MA, Finetti P, Van Egmond M, Matozaki T, Kraal G, Birnbaum D, van Elsas A, Kuijpers TW, Bertucci F, van den Berg TK (2011) CD47-signal regulatory protein- α (SIRP α) interactions form a barrier for antibody-mediated tumor cell destruction. *Proc Natl Acad Sci U S A* 108:18342–18347
- Zhao XW, Matlung HL, Kuijpers TW, van den Berg TK (2012) On the mechanism of CD47 targeting in cancer. *Proc Natl Acad Sci U S A* 109:E2843