CELL CYCLE

Role for known cell cycle proteins beyond proliferation in kidney and non-kidney cells:

The traditional paradigm has been that cell cycle regulatory proteins including cyclins, cyclin-dependent kinase (cdk), and cdk-inhibitors govern whether a cell proliferates or not. Although we reported that this is indeed true in mesangial cells and podocytes, we made several discoveries that showed novel roles for "traditional" cell cycle proteins, beyond their regulation of proliferation.

1) an early discovery was that the cdk-inhibitor p27, which is constitutively expressed in most quiescent cells, also has a major role in regulating cell survival. We showed in mesangial cells and fibroblasts, both in vitro and in vivo, that the threshold for apoptosis was significantly reduced when p27 levels were lowered/absent (*a*). In a follow up series of studies, we showed that the mechanism of this effect was due to an increase in unregulated and unscheduled activity of cdk2 (*b*). When either p27 and or cdk2 was overexpressed, kidney and non-kidney cells have augmented survival in response to stress.
2) we reported how cdk2 might also lead to cell death. Previous studies had shown that cdk2 is nuclear, which is required for its regulation of DNA synthesis through signaling pathways. However, our studies showed that when cdk2 was active, but translocated from the nucleus to the cytoplasm in states of injury, the change in subcellular compartment of active cdk2 switched cells from a proliferative pathway to an apoptotic pathway (*c*). This has lead to one of the mechanistic explanations why proliferating cells are more prone to apoptosis.
3) kidney podocytes and mesangial cells oftentimes undergo an increase in cell size due to hypertrophy. Studies have shown that these are maladaptive responses, leading to further detrimental outcomes. It had been shown that hypertrophy is due to an increase in a cell's protein content relative to its DNA content. We have several publications (*e.g. d*) that a major mechanism of kidney cell hypertrophy induced by diabetes, cytokines and mechanical stretch are the upregulation of the cdk-inhibitors p21 and p27 at a time when cells are late in G1, when intracellular protein synthesis is maximal. By inhibiting DNA synthesis, these cdk-inhibitors send cells down a hypertrophic fate.

(a) Ophascharoensuk V, Fero ML, Hughes J, Roberts JM, **Shankland SJ**. The cyclin-dependent kinase inhibitor p27Kip1 safeguards against inflammatory injury. Nat Med. 1998 May;4(5):575-80. *PMID: 9585231*

(b) Pippin JW, Durvasula R, Petermann A, Hiromura K, Couser WG, **Shankland SJ**. DNA damage is a novel response to sublytic complement C5b-9-induced injury in podocytes. J Clin Invest. 2003 Mar;111(6):877-85. *PMID: 10074476*

(c) Hiromura K1, Pippin JW, Blonski MJ, Roberts JM, **Shankland SJ.** The subcellular localization of cyclin dependent kinase 2 determines the fate of mesangial cells: role in apoptosis and proliferation. Oncogene. 2002 Mar 7;21(11):1750-8. *PMID: 11896606*

(d) Petermann AT1, Pippin J, Durvasula R, Pichler R, Hiromura K, Monkawa T, Couser WG, **Shankland SJ**. Mechanical stretch induces podocyte hypertrophy in vitro. Kidney Int. 2005 Jan;67(1):157-66. *PMID: 15610239*

CELL CYCLE

Identification of novel cell cycle proteins governing survival in kidney and non-kidney cells:

In addition to studying "traditional" cell cycle proteins, we identified cyclin I in several terminally differentiated non-cycling cells including podocytes and neurons. However, the role of cyclin I had not been determined, and any potential cdk binding partner remained elusive. We have contributed to better understanding cyclin I biochemistry and function as follows.

1) we showed that cyclin I sets the apoptotic threshold in terminally differentiated cells by binding to cdk5 (*a*). This is the first cyclin partner reported for cdk5. We elucidated that the signaling pathways and pro-survival proteins regulated by cyclin I-cdk5 were MEK and Bcl-2 respectively.
2) we reported that although non-cyclin p35 also binds to cdk5 in podocytes (*b*), we discovered that maximal cdk5 activity, and thus function, requires both cyclin I and p35 (*c*). Thus, cdk5 has dual activators, where one can compensate for the other, but maximal effect occurs by both cyclin I and p35 acting together. Cdk5 is detected in both the nucleus and cytoplasm, but the determinants of its subcellular localization have not been understood.
3) we showed that the subcellular localization of cdk5 is regulated by cyclin I and p35. Cyclin I keeps cdk5 in the nucleus, while p35 keeps it in the cytoplasm (d).

(a) Brinkkoetter PT, Olivier P, Wu JS, Henderson S, Krofft RD, Pippin JW, Hockenbery D, Roberts JM, **Shankland SJ**. Cyclin I activates Cdk5 and regulates expression of Bcl-2 and Bcl-XL in postmitotic mouse cells. *PMID 19729834. PMCID: 2752065.*

(b) Brinkkoetter PT, Wu JS, Ohse T, Krofft RD, Schermer B, Benzing T, Pippin JW, **Shankland SJ**. p35, the non-cyclin activator of Cdk5, protects podocytes against apoptosis in vitro and in vivo. Kidney Int. 2010 Apr;77(8):690-9. *PMID: 20130526.*

(c) Taniguchi Y1, Pippin JW, Hagmann H, Krofft RD, Chang AM, Zhang J, Terada Y, Brinkkoetter P, **Shankland SJ**. Both cyclin I and p35 are required for maximal survival benefit of cyclin-dependent kinase 5 in kidney podocytes. Am J Physiol Renal Physiol. 2012 May 1;302(9):F1161-71. *PMID: 22262481. PMCID: 3362174.*

(d) Hagmann H, Taniguchi Y, Pippin JW, Kauerz HM, Benzing T, Shankland SJ, Brinkkoetter PT. Cyclin I and p35 determine the subcellular distribution of Cdk5. Am J Physiol Cell Physiol. 2014 Dec 10. [Epub ahead of print] PMID: 25500740