BIOGRAPHICAL SKETCH

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NAME: Chan, Charles K.F.

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POSITION TITLE: Assistant Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of California, Berkeley, CA	B.A.	1999	Molecular Biology
Stanford University, CA	Ph.D.	2011	Developmental Biology

A. Personal Statement

I am an Assistant Professor in the Department of Surgery, Division of Plastic Surgery at Stanford School of Medicine. I am also affiliated with Stanford's Institute of Stem Cell Biology and Regenerative Medicine. My research focuses on the biology of aging in stem cells and stem cell niches. Niches are the highly specialized but poorly understood microenvironments that regulate stem cell activity. Using a reductionist approach, I have pioneered techniques to identify and isolate stem/progenitor cells of individual tissue types, including bone, cartilage, and blood vessels. These basic components can also be combined together to reconstitute a functional bone marrow niche that can support hematopoietic stem cells (HSCs). We recently identified a mouse skeletal stem cell (mSSC) that has the ability to make bone, cartilage, and HSC niches, *in vitro* and *in vivo*. I lead a small group of graduate students and medical fellows on understanding how SSC-HSC interactions in the bone marrow niche changes with age. One aspect that we are investigating is the role of SSC aging in HSC aging. Specifically, we are now investigating if aged SSC generates an aged niche that encourages selection of aged HSC, possibly leading to enhanced osteoclast production that could contribute to osteoporosis in aged bones. We also found that this age-related skewing can be partially reversed by parabiosing aged mice to young mice (manuscript in preparation). To try and reverse aged phenotypes, our group is developing gene-editing approaches to modifying varieties of hematopoietic cells that circulate and home to distinct tissue types, for instance osteoclasts and microglia, to deliver niche factors that stimulate rejuvenation of aged tissues. With these studies as a foundation, we are starting to determine how aging is differentially regulated in mice and humans to facilitate translation of our basic findings.

B. Positions and Honors

Positions:

1990-1991 Research Assistant, California State University Los Angeles, Alan Goldstein Laboratory
(Phosphate solubilizing bacteria for plant growth)

1992-1995 Research Assistant, University of Southern California, S. Ashraf Imam Laboratory
(Antigens to human breast cancer)

1996-1999 Research Assistant, University of California Berkeley, Mark K. Bennett Laboratory
(Structural biology of SNARE membrane fusion complexes)

1999-2002 Research Assistant, University of California Berkeley, Hsiao-Ping Moore Laboratory
(Mechanism of secretory granule formation and secretion)

2002-2011 PhD. Student, Stanford University, Irving Weissman Laboratory
(Aging of the hematopoietic stem cell niche)

2011-2014 Postdoctoral Scholar, Irving Weissman and Michael Longaker Laboratories
(Normal and neoplastic stem cell niches)

2014-2017 Instructor, Irving Weissman and Michael Longaker Laboratories

2017-Present Assistant Professor (University Tenure Line), Department of Surgery, Division of Plastic and Reconstructive Surgery, Stanford University School of Medicine.

Honors:

1995 Edmondson Fellowship. University of Southern California
1999 BA received with Honors from Department of Molecular and Cellular Biology, UC Berkeley
2003 Neonatal Development Training Fellowship, Stanford University
2007 Regenerative Medicine Training Fellowship, Stanford University
2008 Stanford University School of Medicine Biosciences Graduate Education Award for Excellence in Teaching
2011 Finalist. NIH Director's Early Independence Awards (DP5). "Identification of human bone marrow stromal populations and their progenitors."
2011 Siebel Scholar (2011-2013)
2013 Prostate Cancer Foundation Young Investigator Award (2013-2016) "Identification and therapeutic targeting of cancer stem cell niche interactions in metastatic prostate bone marrow disease."
2015 NIH Pathway to Independence Award (K99/R00) (2015-2020) "Mechanisms of Skeletal Stem Cell Aging."

C. Contributions to Science

1. Molecular, Cellular and Structural Biology

My early research relates to the identity, function, and molecular structure of the SNARES "SNAP (Soluble NSF Attachment Protein) REceptor". SNAREs are a family of proteins that mediate trafficking of membrane bound vesicles from one organelle to another within a cell and also from inside a cell to the plasma membrane. They are also the primary molecular machinery for fusing lipid bilayers, which is necessary for vesicle fusion and for protein secretion. My team at Berkeley was the first to solve the structure of this SNARE complex and show how it mediates membrane fusion by "zippering" two adjacent membrane lipid bilayers together. I am now revisiting some of these molecular approaches to design new types of biomolecules that could facilitate specific activation of signaling pathways important in stem cell activity that could drive tissue regeneration or rejuvenation.

1. Poirier, MA, Xiao, W, Macosko, JC, **Chan, C**, Shin, YK, Bennett, MK. The synaptic SNARE complex is a parallel four-stranded helical bundle. ***Nature Structural Biology***. 1998 Sep, 5(9): 765-9

2. Poirier, MA, Hao, JC, Malkus, PN, **Chan, C**; Moore, MF; King, DS; Bennett, MK. Protease resistance of syntaxin, SNAP-25, VAMP complexes. Implications for assembly and structure. ***Journal of Biological Chemistry***. 1998 May 1, 273(18): 11370-7

3. **Chan, C**, Eaton, BA, Moore, HP. Secretory Granules: Methods of Preparation. ***Nature Encyclopedia of Life Sciences.*** 2002

2. Normal and Neoplastic Stem Cell Niches

During my PhD training, I focused on determining how distinct types of tissue-specific stem cells interact with one another in the context of organs to coordinate their regenerative activity. I developed an *in vivo* experimental system to functionally evaluate specific cell types in bones for their ability to maintain and support hematopoietic stem cells (HSC), which forms all blood and immune cells. I succeeded in reassembling a functional hematopoietic stem cell niche from purified bone marrow components at an ectopic site, the renal capsule, which became vascularized by ingressing host endothelial cells and engrafted with circulating host-derived HSC. Using this niche assay as a genetic screen, we identified novel pathways involved in niche formation. Importantly, we find that both HSC and SSC contribute to the generation of niche cells that mediates communication and coordination between hematopoiesis and skeletalgenesis during growth, homeostasis and regeneration. We are now establishing new single cell- methods for elucidating specific skeletal niche defects underlying skeletal disease in diabetes, and aging and how colonization of the bone marrow niche alters the biology of bone micro-environment and contributes to cancer progression

1. **Chan CK***, Chen CC, Luppen CA, Kim JB, DeBoer AT, Wei K, Helms JA, Kuo CJ, Kraft DL, Weissman IL. Endochondral ossification is required for haematopoietic stem-cell niche formation. ***Nature.*** 2009 Jan 22; 457(7228): 490-4. Epub 2008 Dec 10. *Co-Corresponding Author

2. Chao MP, Alizadeh AA, Tang C, Myklebust JH, Varghese B, Gill S, Jan M, Cha AC**, Chan CK**, Tan BT, Park CY, Zhao F, Kohrt HE, Malumbres R, Briones J, Gascoyne RD, Lossos IS, Levy R, Weissman IL, Majeti R. Anti-CD47 antibody synergizes with rituximab to promote phagocytosis and eradicate non- Hodgkin lymphoma. ***Cell***. 2010 Sep 3; 142(5): 699-713 12.

3. Reinisch A, Etchart N, Thomas D, Hofmann NA, Fruehwirth M, Sinha R, **Chan CK**, Senarath-Yapa K, Seo E, Wearda T, Hartwig UF, Beham-Schmid C, Trajanoski S, Lin Q, Wagner W, Dullin C, Alves F, Andreeff M,Weissman IL, Longaker MT, Schallmoser K, Majeti R, Strunk D . Epigenetic and in vivo comparison of diverse MSC sources reveals an endochondral signature for human hematopoietic niche formation. Blood. January 8, 2015

4. **Chan CK***, Lindau P, Jiang W, Chen J, Zhang L, Chen CC, Seita J, Sahoo D, Kim J, Park S, Nag D, Lee AS, Gong Y, Kulkarni S, Luppen C, Theologis A, Wan D, DeBoer A, Vincent-Tompkins JD, Seo EY, Loh K, Walmsley GG, Kraft DL, Wu JC, Longaker MT , & Weissman IL. A Clonal Precursor of Bone, Cartilage, and Hematopoietic Niche Stromal Cells. ***Proc Natl Acad Sci U S A.*** July 15, 2013 *Co-Corresponding Author

3. Skeletal Stem Cells

During my Post-Doctoral and Semi-Independent Investigator phase, I continued tracing the lineages of progenitor cells in mice and humans that could initiate HSC niches. I identified a self-renewing skeletal stem cell in mice (SSCs), which serves as a self-renewing precursor of other progenitors of bone, cartilage and hematopoietic-supportive niche stromal cells. Molecular characterization of the SSC niche then revealed specific signaling pathways guiding SSC expansion and differentiation towards bone, cartilage or stromal lineages.

1. **Chan CK***†, Seo E*, Chen J*, Lo D*, McArdle A, Sinha R, Seita J, Vincent-Thomkins J, Wearda T, Tevlin R, Chung M, Marecic O, Tran MC, Upton RD, Walmsley G, Lee AS, Sahoo D, Kuo CJ, Weissman IL, Longaker MT†. Identification and Specification of mouse Skeletal Stem Cells. Cell. January 15, 2015 *Equal Contributors.†Co-corresponding Author

2. Marecic O, Tevlin R, McArdle A, Seo EY, Wearda T, Duldulao C, Walmsley GG, Nguyen A, Weissman IL*†, **Chan CK***†, Longaker MT*†. Identification and characterization of an injury-induced skeletal progenitor. Proc Natl Acad Sci U S A. 2015 Aug 11;112(32):9920-5. doi: 10.1073/pnas.1513066112. Epub 2015 Jul 27. PMID: 26216955 *Co-Senior. †Co-corresponding Authors.

3. Levi B, Hyun JS, Montoro DT, Lo DD, **Chan CK**, Hu S, Sun N, Lee M, Grova M, Connolly AJ, Wu JC, Gurtner GC, Weissman IL, Wan DC, Longaker MT. In vivo directed differentiation of pluripotent stem cells for skeletal regeneration. ***Proc Natl Acad Sci U S A.*** 2012 Dec 11; 109(50): 20379-84

4. Regenerative Medicine

Stem cells are the source for new cells needed to replenish those lost to injury or disease. I am investigating the microenvironment of stem cells during injury to identify specific combinations of signaling molecules that could be used to stimulate stem cell-mediated regeneration. I am also engineering novel approaches to transplant stem cell niche factors to sites of injury to stimulate regeneration. For instance, I have recently found a way to induce SSC formation in situ using only soluble protein factors and subsequently regulating the SSC niche to specify their differentiation towards bone, cartilage, or stromal cells. We are developing new methods to deliver other types of stem cells such as vasculature stem cells and their niche components to repair injuries that exceed the capacity of resident stem cells to regenerate.

1. **Chan CK***†, Lee AS*, Seo E, Park S, Gong Y, Jiang W, Nag D, Lim S, Lu WJ, Xing W, Weissman IL**†, Wu JC**†, Longaker MT**† Identification of a novel multipotent adipose and vessel progenitor cell from stromal tissue for treatment of ischemic injury. (In Revision, Journal of Clinical Investigation 2016) *Equal Contributors. †Co-corresponding Authors.

2. Zielins ER, Paik K, Ransom RC, Brett EA, Blackshear CP, Luan A, Walmsley GG, Atashroo DA, Senarath-Yapa K, Momeni A, Rennert R, Sorkin M, Seo EY, **Chan CK**, Gurtner GC, Longaker MT,

Wan DC. Enrichment of Adipose-Derived Stromal Cells for BMPR1A Facilitates Enhanced Adipogenesis. Tissue Eng Part A. 2016 Feb;22(3-4):214-21. doi: 10.1089/ten.TEA.2015.0278. PMID: 26585335

3. Tevlin R, McArdle A, Brett E, Chung MT, Paik K, Seo EY, Walmsley GG, Duldulao CR, Atashroo D, Zielins E, Vistnes S, **Chan CK,** Wan DC, Longaker MT. A Novel Method of Human Adipose-Derived Stem Cell Isolation with Resultant Increased Cell Yield. Plast Reconstr Surg. 2016 Dec;138(6):983e-996e. PMID: 27537222

4. Agarwal S, Loder S, Cholok D, Peterson J, Li J, Fireman D, Breuler C, Hsieh HS, Ranganathan K, Hwang C, Drake J, Li S, **Chan CK**, Longaker MT, Levi B. Local and Circulating Endothelial Cells Undergo Endothelial to Mesenchymal Transition (EndMT) in Response to Musculoskeletal Injury. Sci Rep. 2016 Sep 12;6:32514. doi: 10.1038/srep32514. PMID: 27616463

5. Chan CK, Longaker MT. Fibroblasts become fat to reduce scarring. Science. 2017 Feb 17;355(6326):693-694. doi: 10.1126/science.aam6748. No abstract available. PMID: 28209860

5. Stem Cell Aging

Tissue aging could be a manifestation of gradual age-related decline in resident stem cell activity or heterogeneity that alters their ability to regenerate tissues. We are investigating stem cell aging from the perspective of skeletal tissues and its skeletal stem cells (SSC). Overtime, even slight differences in young compared to aged SSC activity could accumulate and contribute to progressive decline in bone tissue integrity and regenerative potential during aging. Since we could isolate and transplant pure SSC, we are investigating the inherent **cell-intrinsic** and external **cell-extrinsic** aspects of mouse and human SSC aging, separately. It has been observed that diabetes mellitus leads to some phenotypes in bones that are associated with aging, such as osteoporosis and deficient bone healing. We find that SSC frequency and activity are diminished in diabetic bones due to deficiencies in hedgehog signaling. Extrinsic delivery of Indian Hedgehog restores this signaling defect and restores SSC mediated bone formation during fracture repair.

1. McArdle A, Seo E, Tevlin R, Marecic O, Li S, Li X, Januszyk M, Seita J, Gurtner GC, Snyder MP, Weissman IL*, Longaker MT*†, **Chan CK***†. Shifting stem cell dynamics on the blood-bone axis underlie skeletal aging. (In Preparation 2017) *Co-Senior. †Co-corresponding Authors.

2. Tevlin R, McArdle A, Seo EY, Tong XM, Li XY, Marecic O, Wearda T, Duldulao CR, Zimdah BJ, Malkovskiy A, Nguyen A, Januszyk M, Rodrigues M, Maan Z, Paik K, Yapa KS, Rajadas J, Wan DC, Gurtner GC, Snyder M, Beachy PA, Yang F, Weissman IL, **Chan CK***, and Longaker MT*. Pharmacological Rescue of Diabetic Skeletal Stem Cell Niches. Sci Transl Med. 2017 Jan 11;9(372). *Co-Senior, Co-corresponding Authors.

3. Chan CK, Ransom RC, Longaker MT. Lectins bring benefits to bones. Elife. 2016 Dec 13;5. pii: e22926. doi: 10.7554/eLife.22926. PMID: 27960074

D. Additional Information: Research Support and/or Scholastic Performance

Research Support

Prostate Cancer Foundation
Young Investigator Award — Chan (PI) — 09/30/13-09/30/16
Characterization of metastatic prostate cancer stem cell niches in the bone marrow.
(My role is to lead a project to determine how metastatic cells can colonize the bone marrow, how they change in this new environment, and how to ablate them once they have engrafted)

National Institute of Health (NIH); Institute: National Institute on Aging (NIA);
Career Transition Award (K99). K99-AG049958-01A1 — Chan (PI) — 09/30/15-09/30/20
Mechanisms of Skeletal Stem Cell Aging

(My role is to lead a group to develop new methods to determine the cause of connective tissue aging from the perspective of age-related changes to the s stem cell niche microenvironment and to develop new therapeutics to reverse stem cell aging.)

Patents Filed:
New U.S. Provisional Application 61/194,880 for “Bone Progenitor Cells, Assays, and Uses Thereof”
First Named Inventor: CHAN, CHARLES

New U.S. Provisional Application 62/101,282 for “Factors and Cells that provide for induction of bone, bone marrow, and cartilage”. First Named Inventor: CHAN, CHARLES

New US Provisional Application 62/380,172 for “Identification and Uses of Vasculature Forming Progenitor Cells and Progenitor Cell Combinations”. First Named Inventor: CHAN, CHARLES