BIOGRAPHICAL SKETCH

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NAME: Halaban, Ruth

eRA COMMONS USERNAME (credential, e.g., agency login): rhalaban

POSITION TITLE: Senior Research Scientist

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
Hebrew University, Rehovot, Israel	B.Sc.	07/1962	Biology
Hebrew University, Rehovot, Israel	M.Sc.	07/1964	Genetics
Princeton University, Princeton, NJ	Ph.D.	07/1968	Biology

A. Personal Statement

As the past Director of the Yale SPORE in Skin Cancer (YSPORE) for 11 years, and the Co-Director of Core 2, Biospecimen Resource Core, I have coordinated several studies with investigators on melanoma research and provided funds as well as specimens and reagents to investigators for different projects. I have established operating procedures for the collection of tumors, tissues, blood, normal skin, nevi and clinical information, growing melanoma cells and normal lymphocytes in culture for multiple purposes, such as protein analyses, DNA extraction and sequence analysis of novel mutations. These samples are shared with investigators at Yale, other Skin SPOREs, nationally and around the world. My main goals as the Biospecimen Resource Core Director are to continue these activities, to foster collaboration between investigators at Yale and outside institutions, and to ensure that the molecular and clinical information becomes available upon request. My research goals are: 1) to test novel mutations and genomic aberrations in melanoma that promote tumor initiation and metastasis; 2) to understand the underlying molecular mechanisms that lead to melanoma resistance to therapy; 3) to identify new targets for therapy; and 4) to promote the application of molecular diagnosis of melanoma in the clinic. I have trained numerous pre- and post-doctoral fellows and have worked within the department of Dermatology to enhance research programs.

Awards

Award for Lifetime Achievement in Melanoma research, Society of Melanoma Research (SMR), 2009
Myron Gordon Award, The International Federation of Pigment Cell Societies (IFPCS), 2011
Basic Research Award, Yale Cancer Center, 2012
Research Achievement Award in Melanoma and Skin Cancer, American Skin Association, 2017
Lifetime Achievement Award, Yale Cancer Center, 2020

Recent publications

Earland N, Zhang W, Usmani A, Nene A, Bacchiocchi A, Chen DY, Sznol M, Halaban R, Chaudhuri AA, Newman AM. CD4 T cells and toxicity from immune checkpoint blockade. Immunol Rev, 2023, 318: 96-109, PMID: 37491734

Lozano AX, Chaudhuri AA, Nene A, Bacchiocchi A, Earland N, Vesely MD, Usmani A, Turner BE, Steen CB, Luca BA, Badri T, Gulati GS, Vahid MR, Khameneh F, Harris PK, Chen DY, Dhodapkar K, Sznol M, Halaban R, Newman AM**.** T cell characteristics associated with toxicity to immune checkpoint blockade in patients with melanoma. Nature Med, 2022, 28: 353-362**.** PMID: 35027754

Farshidfar F, Rhrissorrakrai K, Levovitz C, Peng C, Knight J, Bacchiocchi A, Su J, Yin M, Sznol M, Ariyan S, Clune J, Olino K, Parida L, Nikolaus J, Zhang M, Zhao S, Wang Y, Huang G, Wan M, Li X, Cao J, Yan Q,

Chen X, Newman AM, Halaban R. Integrative molecular and clinical profiling of acral melanoma links focal amplification of 22q11.21 to metastasis. Nat Commun, 2022, 13: 898. PMC9090916

Das R, Bar N, Ferreira M, Newman AM, Zhang L, Bailur JK, Bacchiocchi A, Kluger H, Wei W, Halaban R, Sznol M, Dhodapkar MV, Dhodapkar KM. Early B cell changes predict autoimmunity following combination immune checkpoint blockade. J Clinic Invest, 2018, 128: 715-720. PMID: 29309048

Sanmamed MF, Perez-Gracia JL, Schalper KA, Fusco JP, Gonzalez A, Rodriguez-Ruiz ME, Oñate C, Perez G, Alfaro C, Martín-Algarra S, Andueza MP, Gurpide A, Morgado M, Wang J, Bacchiocchi A, Halaban R, Kluger H, Chen L, Sznol M, Melero I. Changes in serum interleukin-8 (IL-8) levels reflect and predict response to anti-PD-1 treatment in melanoma and non-small-cell lung cancer patients. Ann Oncol, 2017, 28:1988-1995. PMID: 28595336

B. Positions, Scientific Appointments and Honors

Positions and Employment

2015-current, Co-Director, the Biospecimen Core, Yale SPORE in Skin Cancer, Yale University School of Medicine, New Haven, CT
2004-2015 Director, Yale SPORE in Skin Cancer, Yale University School of Medicine
1992-2004 Director, Cell Culture Core, co-director Yale Skin Diseases Research Core Center, Dermatology Department, Yale University School of Medicine, New Haven CT
1971-1973 Research Associate, Biology Department, State University of New York at Albany
1973- Advanced through the ranks to the current position of Professor/Senior Research Scientist, Yale University School of Medicine, Department of Dermatology, New Haven, CT
1968-1969 Instructor, Biology Department, Princeton University, Princeton, NJ
1969-1971 Research Associate, Biology Department, Brookhaven National Laboratory, Upton, New York

Honors

2020 Life Achievement Award, Yale Cancer Center
2017 American Skin Association Research Achievement Award in Melanoma and Skin Cancer
2011 The International Federation of Pigment Cell Societies (IFPCS) Myron Gordon medal
2009 Lifetime Achievement in Melanoma Research award by the Society of Melanoma Research
1998 American Skin Association award for meritorious contribution in the field of melanocyte biology
1985; 2004 Ruth Estrin Memorial Awards for Cancer Research
1976; 2005 Swebilius Cancer Research Awards

C. Contribution to Science (out of 151 publications)

1. Melanocyte biology. We identified the growth factors requirements for normal human melanocytes and the role of bFGF in transformation to melanomas. We were the first to clone the melanocytes specific genes tyrosinase (TYR) and pMEL17/silver and showed that mutations in TYR are the cause of albinism. We were the first to demonstrate the path of glycosylation and degradation of tyrosinase, which lead to understanding of the process of loss of pigmentation in albino melanocytes and in melanoma cells. As a consequence of these accomplishments, we are currently one of the major sources for cultures of normal human and mouse melanocytes, as well as melanoma cells from individual patients. We deposited our mammalian expression vectors for tyrosinase in Addgene, which are provided to every requesting investigator.

    a) **Halaban R**, Ghosh S, Baird A (1987) bFGF is the putative natural growth factor for human melanocytes. In Vitro Cell Dev Biol 23: 47-52. PMID: 3027025
    b) Kwon BS, Haq AK, Pomerantz SH, Halaban R (1987) Isolation and sequence of a cDNA clone for human tyrosinase that maps at the mouse c-albino locus. Proc Natl Acad Sci USA 84: 7473-7477. PMC299318
    c) **Halaban R**, Moellmann G, Tamura A, Kwon BS, Kuklinska E, Pomerantz SH, Lerner AB (1988) Tyrosinases of murine melanocytes with mutations at the albino locus. Proc Natl Acad Sci USA 85: 7241-7245. PMC282161
    d) **Halaban R**, Patton RS, Cheng E, Svedine S, Trombetta ES, Wahl ML, Ariyan S, Hebert DN (2002) Abnormal acidification of melanoma cells induces tyrosinase retention in the early secretory pathway. J Biol Chem 277: 14821-14828. DOI:10.1074/jbc.M111497200

2. Mechanism of malignant transformation of human melanocytes to melanoma. We were the first to show that some of the major events are aberrant expression of bFGF, aberrant gene expression and epigenetic modification of DNA. Our global gene expression data revealed the following: (a) activation of the NOTCH pathway; (b) altered expression of transcriptional regulators implicated in embryonic development; (c) coordinated activation of cancer/testis antigens; (d) coordinated down-regulation of several immune modulation genes, in particular in the IFN pathways; (e) down-regulation of several genes implicated in membrane trafficking events; and (f) down-regulation of growth suppressors, such as the Prader-Willi gene NECDIN, whose function was confirmed by overexpression of ectopic Flag-necdin. Our epigenetic studies showed that some of these genes are regulated by DNA methylation.

   a) **Halaban R**, Kwon BS, Ghosh S, Delli Bovi P, Baird A (1988) bFGF as an autocrine growth factor for human melanomas. Oncogene Research 3: 177-186. PMID: 3226725
   b) Hoek K, Rimm DL, Williams KR, Zhao H, Ariyan S, Lin A, Kluger HM, Berger AJ, Cheng E, Trombetta ES, Wu T, Niinobe M, Yoshikawa K, Hannigan GE, **Halaban R** (2004) Expression profiling reveals novel pathways in the transformation of melanocytes to melanomas. Cancer Res 64: 5270-5282. PMID: 1528933310.1158/0008-5472.CAN-04-0731
   c) **Halaban R**, Krauthammer M, Pelizzola M, Cheng E, Kovacs D, Sznol M, Ariyan S, Narayan D, Bacchiocchi A, Molinaro A, Kluger Y, Deng M, Tran N, Zhang W, Picardo M, Enghild JJ (2009) Integrative analysis of epigenetic modulation in melanoma cell response to decitabine: clinical implications. PLoS ONE 4: e4563. PMC2642998
   d) Koga Y, Pelizzola M, Cheng E, Krauthammer M, Sznol M, Ariyan S, Narayan D, Molinaro AM, **Halaban R**, Weissman SM (2009) Genome-wide screen of promoter methylation identifies novel markers in melanoma. Genome Res 19: 1462-1470. PMC2720187

3. Drug responses and drug resistance. We were among the first to identify the “paradoxical” effect of PLX4032 (Vemurafenib). Our studies with patient-derived melanoma cells showed that, paradoxically, while PLX4032 inhibited ERK1/2 in the highly sensitive $BRAF^{V600E/K}$, it activated the pathway in the resistant $BRAF^{WT}$ cells, via RAF1 activation, regardless of the status of mutations in NRAS or PTEN. The persistently active ERK1/2 triggered downstream effectors in $BRAF^{WT}$ melanoma cells and induced changes in the expression of a wide spectrum of genes associated with cell cycle control. Furthermore, PLX4032 increased the rate of proliferation of growth factor dependent $NRAS^{Q61L}$ mutant primary melanoma cells, reduced cell adherence and increased mobility in of cells from advanced lesions. The results suggest that the drug can confer an advantage to $BRAF^{WT}$ primary and metastatic tumor cells in vivo and provide markers for monitoring clinical responses. Indeed the “paradoxical effect of vemurafenib” explains now the emergence of cutaneous squamous-cell carcinomas (SCCs) and secondary melanomas in patients on BRAF-inhibitor therapy and recent reports demonstrated an effect on T-cell phenotype resulting in improved functionality in vivo (Ribas and colleagues). In the drug resistance field, we performed whole-exome sequencing and discovered a novel BRAF mutation that confers resistance to PLX4032 of drug-resistant $BRAF^{V600K}$ melanoma cells. The novel BRAF mutation, a L505H is the first resistance-conferring second-site mutation identified in BRAF mutant cells. This mutation was also identified in human prostate cancer. We also ruled out that mutation in MEK1 confers drug resistance.

   a) **Halaban R**, Zhang W, Bacchiocchi A, Cheng E, Parisi F, Ariyan S, Krauthammer M, McCusker JP, Kluger Y, Sznol M (2010) PLX4032, a selective BRAF(V600E) kinase inhibitor, activates the ERK pathway and enhances cell migration and proliferation of BRAF(WT) melanoma cells. Pigment Cell Melanoma Res 23: 190-200. PMC2848976
   b) Choi J, Landrette SF, Wang T, Evans P, Bacchiocchi A, Bjornson R, Cheng E, Stiegler AL, Gathiaka S, Acevedo O, Boggon TJ, Krauthammer M, **Halaban R**, Xu T (2014) Identification of PLX4032-resistance mechanisms and implications for novel RAF inhibitors. Pigment Cell Melanoma Res 27: 253-262. PMC4065135
   c) Shi H, Moriceau G, Kong X, Koya RC, Nazarian R, Pupo GM, Bacchiocchi A, Dahlman KB, Chmielowski B, Sosman JA, **Halaban R**, Kefford RF, Long GV, Ribas A, Lo RS (2012) Preexisting MEK1 Exon 3 mutations in V600E/KBRAF melanomas do not confer resistance to BRAF inhibitors. Cancer Discovery 2: 414-424. PMC3594852.

4. Novel mutations and genomic aberrations discovered by whole exome-capture sequencing.

We characterized the mutational landscape of over 300 melanomas. Notably, we identified a recurrent UV-signature, activating mutation in RAC1 in 5% of sun-exposed melanomas (P29S). Crystal structures, and biochemical and functional studies of RAC1P29S showed that the alteration releases the conformational restraint conferred by the conserved proline, causes an increased binding of the protein to downstream effectors, and promotes melanocyte proliferation and migration. We also demonstrated that the activated melanoma RAC1P29S protein maintains intrinsic GTP hydrolysis and is spontaneously activated by substantially increased inherent GDP/GTP nucleotide exchange. We also showed that NF1, a negative regulator of RAS, is the 3rd most frequently mutated gene in melanoma, after BRAF and NRAS. Inactivating NF1 mutations were present in 46% of BRAF/RAS wild-type melanomas, occurred in older patients, and showed a distinct co-mutation pattern with other RASopathy genes, particularly RASA2. Functional studies demonstrated that NF1 suppression leads to increased RAS activation in most, but not all melanoma cases. We showed that germline MC1R status influences somatic mutation burden in melanoma (Robles-Espinoza, et al, 2016, Nat Commun 7, 12064. PMC49458740). We showed that melanin may be carcinogenic as well as protective against cancer (Premi S et al. Science 347: 842-847. PMC4432913)

We characterized 104 acral melanoma tumors and found that late-arising focal amplifications in chr22q11.21 are associated with poor outcome and regional metastasis. Within chr22q11.21, we identified two genes, *LZTR1* (leucine zipper like transcription regulator 1) and *CRKL* (CRK like proto-oncogene, adaptor protein), as key candidate drivers of regional metastasis. Downregulation of LZTR1, inhibited melanoma cell proliferation, and overexpression of *LZTR1* or *CRKL* in normal human melanocytes facilitated anchorage-independent growth by reducing E-cadherin, increasing N-cadherin, and activating integrin □1.

a) Krauthammer M, Kong Y, Ha BH, Evans P, Bacchiocchi A, McCusker JP, Cheng E, Davis MJ, Goh G, Choi M, Ariyan S, Narayan D, Dutton-Regester K, Capatana A, Holman EC, Bosenberg M, Sznol M, Kluger HM, Brash DE, Stern DF, Materin MA, Lo RS, Mane S, Ma S, Kidd KK, Hayward NK, Lifton RP, Schlessinger J, Boggon TJ, **Halaban R** (2012) Exome sequencing identifies recurrent somatic RAC1 mutations in melanoma. Nat Genet 44: 1006-1014. PMC3432702
b) Krauthammer, M., Y. Kong, A. Bacchiocchi, P. Evans, N. Pornputtapong, C. Wu, J.P. McCusker, S. Ma, E. Cheng, R. Straub, M. Serin, M. Bosenberg, S. Ariyan, D. Narayan, M. Sznol, H.M. Kluger, S. Mane, J. Schlessinger, R.P. Lifton, and **R. Halaban**. 2015. Exome sequencing identifies recurrent mutations in NF1 and RASopathy genes in sun-exposed melanomas. Nat Genet. 47: 996-1002. PMC4916843
c) Lazova, R., Pornputtapong, N., **Halaban, R.**, Bosenberg, M., Bai, Y., Chai, H., and Krauthammer, M. 2017. Spitz nevi and Spitzoid melanomas: exome sequencing and comparison with conventional melanocytic nevi and melanomas. Mod Pathol, 30, 640-649. PMC5413430
d) Farshidfar, F., Rhrissorrakrai, K., Levovitz, C., Peng, C., Knight, J., Bacchiocchi, A., Su, J., Yin, M., Sznol, M., Ariyan, S., Clune, J., Olino, K., Parida, L., Nikolaus, J., Zhang, M., Zhao, S., Wang, Y., Huang, G., Wan, M., Li, X., Cao, J., Yan, Q., Chen, X., Newman, A. M., and **Halaban, R.** (2022) Integrative molecular and clinical profiling of acral melanoma links focal amplification of 22q11.21 to metastasis. Nat Commun 13, 2704.PMC8866401

5. Circulating immune markers of immunotherapy response and toxicity.

My studies on this subject have been in collaboration with several investigators from Yale and investigators from other institutions. With Drs. Newman, Chaudhuri, Sznol and Chen, we systematically evaluate immunological features in the peripheral blood associated with immune checkpoint inhibitors (ICI)-induced toxicity in patients with metastatic melanoma. We identified common T cell features linked to the development of severe irAEs within three months of treatment initiation. We found that two pretreatment factors in circulation — abundance of activated CD4 memory T cells and TCR diversity — are associated with severe irAE development regardless of organ system involvement. These features were independent of key clinical variables, including durable clinical response and treatment with anti-PD-1 monotherapy or anti-PD-1 and anti-CTLA-4 combination therapy. Leveraging these findings, we developed predictive models of irAE development and explored their utility for pretreatment and early on-treatment identification of ICI-induced toxicity. Our data, published in *Nature Medicine*, and using samples from my biospecimen repository, form the basis for the current R01 grant proposal. We have also had extensive collaborations with Dr. Dhodapkar, which resulted in several publications. For example, we found that early changes in B cells following combination

immunotherapy is associated with increased irAE risk. We have also worked on immunotherapy response, showing that changes in serum IL-8 levels reflect response to anti-PD1 immunotherapy in melanoma and non-small-cell lung cancer patients.

- Lozano, A. X., Chaudhuri, A. A., Nene, A., Bacchiocchi, A., Earland, N., Vesely, M. D., Usmani, A., Turner, B. E., Steen, C. B., Luca, B. A., Badri, T., Gulati, G. S., Vahid, M. R., Khameneh, F., Harris, P. K., Chen, D. Y., Dhodapkar, K., Sznol, M., Halaban, R., and Newman, A. M. (2022) T cell characteristics associated with toxicity to immune checkpoint blockade in patients with melanoma. Nat Med 28, 353-362, PMCID: PMC8866214
- Das, R., Bar, N., Ferreira, M., Newman, A. M., Zhang, L., Bailur, J. K., Bacchiocchi, A., Kluger, H., Wei, W., Halaban, R., Sznol, M., Dhodapkar, M. V., and Dhodapkar, K. M. (2018) Early B cell changes predict autoimmunity following combination immune checkpoint blockade. J Clin Invest 128, 715-720, PMC5785243
- Sanmamed, M. F., Perez-Gracia, J. L., Schalper, K. A., Fusco, J. P., Gonzalez, A., Rodriguez-Ruiz, M. E., Oñate, C., Perez, G., Alfaro, C., Martin-Algarra, S., Andueza, M. P., Gurpide, A., Morgado, M., Wang, J., Bacchiocchi, A., Halaban, R., Kluger, H., Chen, L., Sznol, M., Melero, I. (2017) Changes in serum interleukin-8 (IL-8) levels reflect and predict response to anti-PD-1 treatment in melanoma and non-small-cell lung cancer patients. Annals of Oncology 28, 1988-1995. PMC5834104
- Earland, N., Zhang, W., Usmani, A., Nene, A., Bacchiocchi, A., Chen, D. Y., Sznol, M., Halaban, R., Chaudhuri, A. A., Newman, A. M. CD4 T cells and toxicity from immune checkpoint blockade. Immunological Reviews. 2023, 318: 96-109, PMID: 37491734

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