Participation of the lipoprotein receptor LRP1 in hypoxia-HSP90α autocrine signaling to promote keratinocyte migration

David T. Woodley[1], Jianhua Fan[1], Chieh-Fang Cheng[1], Yong Li[1,*], Mei Chen[1], Guojun Bu[2] and Wei Li[1,‡]

[1]Department of Dermatology and the USC-Norris Comprehensive Cancer Center, the University of Southern California Keck School of Medicine, Los Angeles, CA 90033, USA
[2]The Department of Pediatrics, Washington University School of Medicine, St Louis, MO 63130, USA
*Present address: Department of Plastic Surgery, Xijing Hospital, FMMU, China
‡Author for correspondence (e-mail: wli@usc.edu)

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Summary

Hypoxia is a microenvironmental stress in many pathological conditions, including wound healing and tumor invasion. Under hypoxia, the cells are forced to adapt alternative and self-supporting mechanisms. Understanding these mechanisms may lead to new insights into human disorders. We report here a novel autocrine signaling mechanism by which hypoxia promotes human keratinocyte (HK) migration. First, hypoxia triggers HKs to secrete heat shock protein 90-alpha (HSP90α) via a HIF1-dependent pathway. The secreted HSP90α in turn promotes migration, but not proliferation, of the cells. Disruption of the secretion or extracellular function of HSP90α blocked hypoxia-stimulated HK migration. The ubiquitously expressed surface receptor, LRP1 (LDL-receptor-related protein 1), mediates the HSP90α signaling. Inhibition of LRP1 binding to extracellular HSP90α by neutralizing antibodies or genetic silencing of the LRP1 receptor by RNAi completely nullified hypoxia-driven HK migration. Finally, re-introducing a RNAi-resistant LRP1 cDNA into LRP1-downregulated HKs rescued the motogenic response of the cells to hypoxia. We propose that the hypoxia-HSP90α-LRP1 autocrine loop provides previously unrecognized therapeutic targets for human disorders such as chronic wounds and cancer invasion.

Key words: Keratinocytes, Hypoxia, HSP90α, LRP1, Cell motility

Introduction

The microenvironment of wounded skin is hypoxic because of vascular disruption and high oxygen consumption by cells in the wound (Hunt et al., 1972). To adapt to the hypoxic environment, the cells activate a number of novel signaling pathways to induce synthesis and secretion of a wide variety of gene products such as growth factors and extracellular matrices (ECMs). The new gene expression presumably achieves a temporary self-support status for continued cell survival in the absence of an adequate blood supply. One of the most-studied signaling pathways in cells under hypoxia is the hypoxia inducible factor 1 (HIF1)-dependent pathway (Semenza, 2003). HIF1 is a ubiquitously expressed heterodimeric transcription factor that consists of α- and β-subunits and a key regulator of cellular oxygen homeostasis (Semenza, 2000).

Hypoxia promotes migration of human keratinocytes (HKs) (O'Toole et al., 1997; Xia et al., 2001) and dermal fibroblasts (Mogford et al., 2002; Lerman et al., 2003; Li et al., 2007). Acute hypoxia is probably a driving force during skin wound healing (Tandara and Mustoe, 2004). We demonstrated that hypoxia triggers human dermal fibroblasts to secrete heat shock protein 90-alpha (HSP90α), which in turn stimulates cell migration (Li et al., 2007). In the current study, we report a novel autocrine loop that hypoxia uses to promote HK migration.

Results and Discussion

HIF1 is critical for hypoxia's pro-motility signaling in HKs

Using an established HK model to study hypoxia-induced cell motility (O'Tool et al., 1997), we wished to identify the key pathway for hypoxia-driven HK migration. Using the single-cell-based colloidal gold migration assay as shown in Fig. 1A, we observed that hypoxia stimulated HK migration (compare panels b and c). Similar results were obtained using the cell-population-based in vitro wound-healing assay, namely hypoxia significantly enhanced HK migration (Fig. 1B, panels b,c).

We then studied whether induction of HIF1α is necessary and/or sufficient to mediate the effect of hypoxia on motility. First, in hypoxic HKs, we detected a dramatic accumulation of HIF1α protein in a time-dependent fashion (Fig. 1C, panel a). The maximum accumulation of HIF1α appeared to occur after 3 hours under 1% O_2 (lane 3). By contrast, duplicate HK cultures under normoxia (20% O_2) showed no detectable HIF1α (Fig. 1C, panel c).

Second, we constructed the following cDNAs: (1) wt HIF1, (2) a constitutively activated (non-degradable) HIF1 (HIF1αCA5) and (3) a dominant negative HIF1 (HIF1αDN) (Jiang et al., 1996; Kelly et al., 2003) into the lentiviral vector, pRRLsin.MCS-Deco. This system offers more than 90% gene transduction efficiency in primary HKs (Cheng et al., 2008). Following infection, expression of these exogenous HIF1 genes in HKs was confirmed by anti-HIF1 antibody immunoblot analyses. As shown in Fig. 1D, both the wt HIF1 and HIF1αCA5 proteins were detected even under normoxia (lanes 2 and 3). By contrast, endogenous HIF1 was undetectable (panel a, lane 1). Since the HIF1αDN mutant lacks the region amino acids 391-826 that contains the epitopes for anti-HIF1α antibodies (lane 4), we used anti-HA-tag antibodies to confirm the expression of the HA-tagged HIF1αDN. As shown in Fig. 1E, an expected 36 kDa protein species was detected in HIF1αDN-infected HKs (lane 2), but not in vector-infected cells (lane 1). Infection with an HA-tagged Nck cDNA served as a control for the anti-HA antibodies (lane 3).

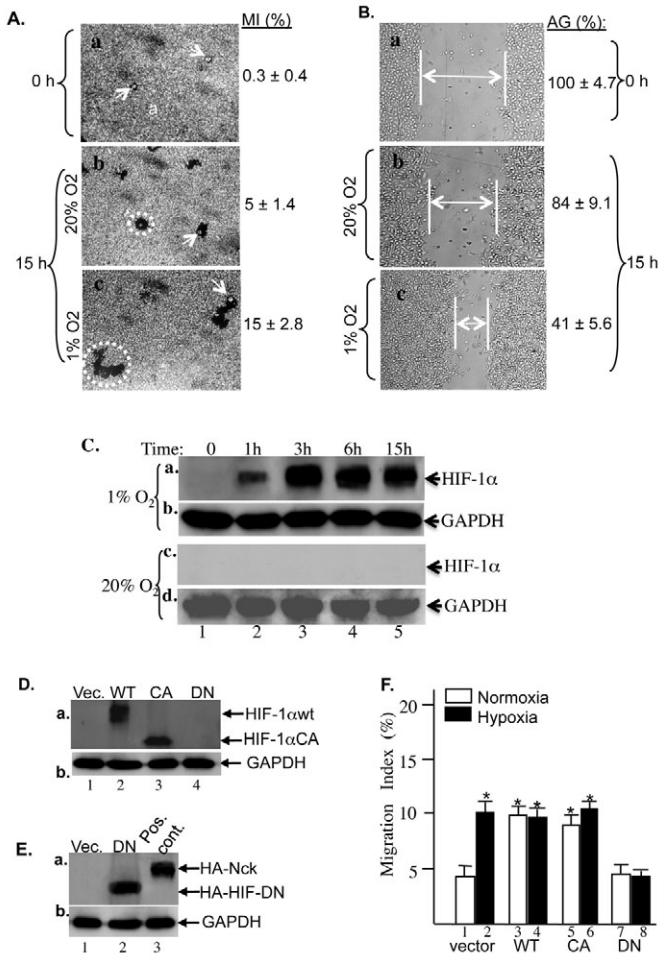

Fig. 1. Hypoxia promotes HK migration through the action of HIF1. HKs were serum-starved and subjected to two cell migration assays (both 15 hours). (A) Colloidal gold migration assay. Representative images of cell migration tracks are shown together with the migration index (MI). An average migration track under each experimental condition is highlighted with a dotted circle for visual purpose only. (B) The in vitro wound healing assay. Cell migrations were photographed and the remaining cell-free space was quantified as the average gap (AG; double-headed arrows) (Li et al., 2004). Values are the means ± s.e.m. of three independent experiments. (C) Lysates of the cells, which were subjected to hypoxia (1% O_2) or normoxia (20% O_2) for the indicated time, were analyzed by western immunoblotting analysis using antibodies specifically against HIF1α (panels a and c). Anti-GAPDH antibody blotting of duplicate membranes was used as a sample loading control (panels b and d). (D,E) HKs were infected with lentivirus-carrying vector (Vec.), wild-type HIF1α (WT), HIF1αCA (CA) and HIF1αDN (DN). 48 hours following infection, the lysates of the cells were immunoblotted with anti-HIF1α antibodies (D, panel a) or anti-HA tag antibody (E, panel a). Anti-GAPDH antibody was used as a sample loading and densitometry scan control (panel b). (F) The HKs carrying vector or HIF1αWT or HIF1αCA or HIF1αDN were subjected to colloidal gold migration assays under either hypoxia (1% O_2) or normoxia (20% O_2). The computer-assistant quantification of the migration is shown as a migration index, as previously described. *Statistically significantly different (P<0.01) from normoxia (20% oxygen). The experiment was carried out four times.

The cells expressing vector alone, HIF1αWT, HIF1αCA or HIF1αDN were then subjected to colloidal gold migration assays under either normoxia or hypoxia in the absence of any exogenously added growth factors. As shown in Fig. 1F, HIF1αWT- or HIF1αCA-overexpressing HKs migrated further than the vector control cells, even under normoxia (bars 3 and 5 versus bar 1), similar to the effect of hypoxia on the parental cells (bar 2). Hypoxia increased migration of control cells as expected (bar 2), but not the maximally stimulated HKs expressing HIF1αWT or HIF1αCA gene (bars 4 and 6). By contrast, expression of the HIF1αDN blocked hypoxia-stimulated migration (bar 8 versus lane 7). These data demonstrated that HIF1 is necessary and sufficient to mediate the entire hypoxia pro-motility signaling.

Hypoxia promotes HK migration via secretion and extracellular function of HSP90α

Hypoxia triggers cells to secrete HSP90α into the extracellular environment, which in turn promotes cell migration (Li et al., 2007). An important question is whether disruption of (1) the secretion of HSP90α or (2) the extracellular function of HSP90α would block hypoxia-induced cell migration. As shown in Fig. 2A, hypoxia and HIF1 expression caused HKs to secrete HSP90α into the culture medium. Equal quantities (see figure legend for details) of HK-conditioned media were immunoblotted with anti-HSP90α antibodies, HSP90α proteins were clearly detected in the preparations from hypoxic (lane 2), but very little from those of normoxic (lane 1) HKs. Expression of the HIF1αCA mutant showed a slightly increased secretion of HSP90α, even under normoxia (lanes 5). By contrast, the dominant-negative HIF1 mutant, HIF1αDN, blocked HSP90α secretion (lanes 3 and 4).

We used two independent approaches to study the role of secreted HSP90α in hypoxia signaling. First, since HSP90α secretion is mediated by the unconventional exosomal trafficking pathway (Hegmans et al., 2004; Clayton et al., 2005; Yu et al., 2006), we investigated whether inhibition of the exosome-mediated protein trafficking affects hypoxia-stimulated HK migration. Dimethyl amiloride (DMA), a specific inhibitor of the exosomal trafficking pathway, inhibited hypoxia-stimulated HK migration in a dose-dependent manner (Fig. 2B, bars 4-7 versus bar 2). By contrast, brefeldin A (BFA), a specific inhibitor of the classical endoplasmic reticulum/Golgi trafficking pathway, showed little inhibition of hypoxia-driven HK migration (Fig. 2B, bar 3). We also used an anti-HSP90α neutralizing antibody to specifically block the function of extracellular HSP90α, since antibodies do not have access to intracellular HSP90α. As shown in Fig. 2C, the presence of the antibody, but not an IgG control, inhibited hypoxia-stimulated HK migration in a dose-dependent fashion (Fig. 2C bars 4-7 versus bars 1 and 3). These results demonstrate that hypoxia-triggered secretion and extracellular function of HSP90α are required for hypoxia-driven HK migration.

LRP1 is essential for both hypoxia- and HSP90α-driven HK migration

LRP1 (LDL receptor-related protein 1) is known as a common receptor for a variety of heat shock proteins, including extracellular HSP90α (Basu et al., 2001). Therefore, we speculated that hypoxia creates an 'HSP90α-LRP1 autocrine loop' to support cell migration. LRP1 consists of a 515 kDa extracellular subunit and a membrane-anchoring 85 kDa subunit, two proteolytic products from a common 600 kDa precursor (Strickland et al., 1990). We undertook three independent approaches to assess the importance of LRP1: (1) we used a neutralizing antibody against the extracellular ligand binding domain of LRP1 to block HSP90α binding to LRP1; (2) we used the lentiviral FG12 system to express two independent siRNAs to downregulate the endogenous LRP1 to an undetectable level; and (3) we re-introduced a siRNA-resistant LRP1 receptor cDNA into LRP1-downregulated HKs to rescue the responsiveness of the cells

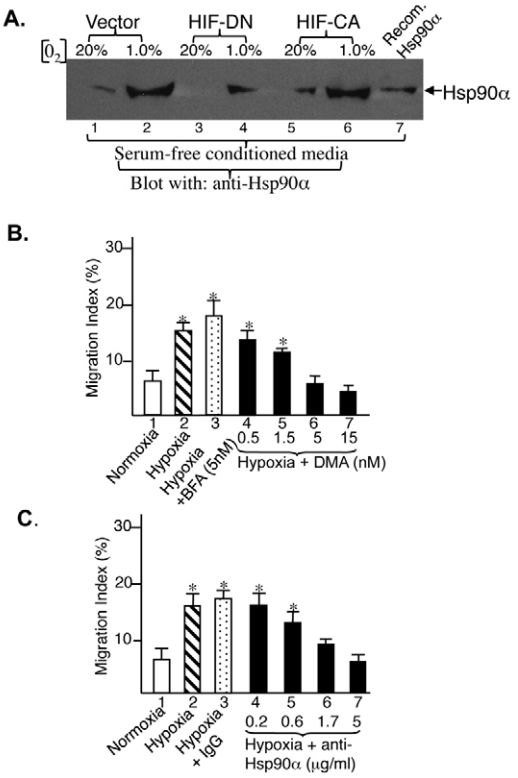

Fig. 2. Hypoxia-triggered and HIF1-mediated secretion of HSP90α is essential for hypoxia-induce HK migration. (A) The HIF1α-engineered HKs were cultured in serum-free medium under either hypoxia (1% O_2) or normoxia (20% O_2) for 15 hours. Cell-free conditioned medium (CM) was collected from each culture condition, concentrated and analyzed by western blot analysis with an anti-HSP90α antibody. Equal loading of these samples was based on two parameters: (1) equal volumes of medium were added to each cell culture plate and (2) the volumes of loaded samples were further calibrated according to the cell counts. Recombinant HSP90α (100 ng) was included as the positive control (lane 7). (B) HKs were subjected to colloidal gold migration assays either under normoxia (20% O_2) or hypoxia (1% O_2) in the absence or presence of the indicated concentrations of dimethyl amiloride (DMA) or brefeldin A (BFA) for 15 hours. Only MIs are shown, as previously described. Values are means ± s.e.m. of the MIs from four independent experiments. *Statistically significantly different (P<0.03) from the controls under the same experimental condition (i.e. bar 1). (C) HKs were subjected to colloidal gold migration assays under hypoxia or normoxia in the absence (bars 1 and 2) or presence of 5 μg of control IgG (bar 3) or increasing amounts of anti-HSP90α neutralizing antibodies (SPS-771; bars 4-7). Values are means ± s.e.m. of the MIs from four independent experiments. *Statistically significantly different (P<0.0%) from their controls under the same experimental condition (bar 1).

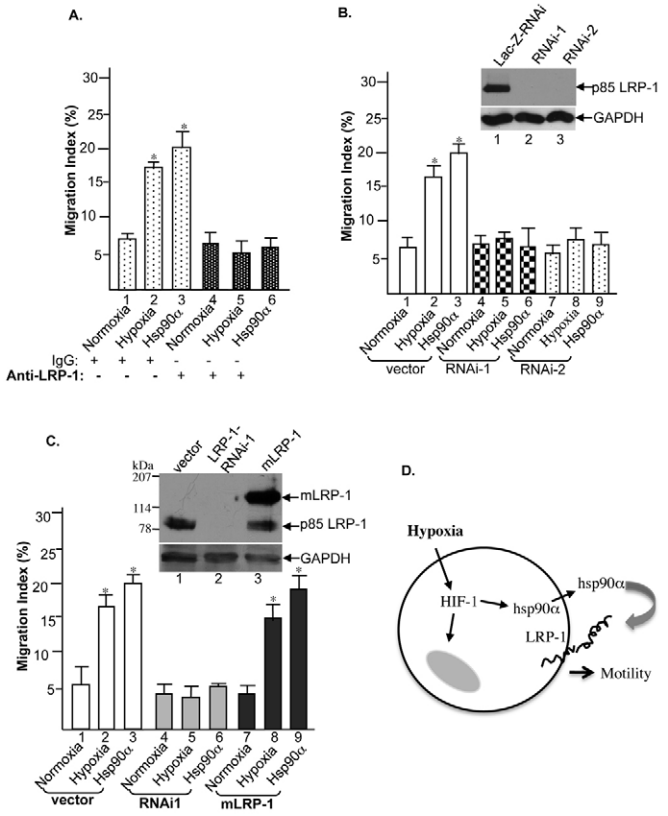

Fig. 3. LRP1 mediates autocrine HSP90α to promote HK migration. (A) The effect of anti-LRP1 neutralizing antibody on HK migration in response to hypoxia or HSP90α. HKs were subjected to colloidal gold migration assays in the presence of either a control IgG or increasing concentrations of an anti-LRP1 neutralizing antibody under hypoxia. (B) Lysates of HKs infected with lentivirus carrying either a control siRNA (*lacZ*-siRNA) or two siRNAs against LRP1 (RNAi-1 and RNAi-2) were analyzed by western blot with an anti-LRP1 antibody (inset). Colloidal gold migration assays were used to assess the response of the cells to hypoxia or HSP90α (10 μg/ml). (C) Lysates of HKs expressing vector alone, siRNA-1 or siRNA-1 plus a rescuing LRP1 mutant (mlRLP1) were analyzed by western blot with an anti-LRP1 antibody, which detected the p85 subunit of LRP1 and the p150 mLRP1 mutant (Inset). The HKs were then subjected to colloidal gold migration assays. *Statistically significant (P<0.03-0.05) over serum-free controls, n=3 or 4. (D) Hypoxia drives HSP90α secretion. Secreted HSP90α binds LRP1 and promotes migration of HKs (and probably the surrounding LRP1-positive dermal cells, as well, during wound healing). The mechanism, by which hypoxia causes HKs to secrete intracellular HSP90α, remains to be determined.

to hypoxia. Hypoxia and HSP90α strongly stimulated HK migration (Fig. 3A, bars 2 and 3 versus bar 1) as expected. Control IgG showed little effect (Fig. 3A, bars 2 and 3). However, the addition of anti-LRP1 antibodies completely blocked both hypoxia-induced (Fig. 3A, bar 5 versus bar 2) and HSP90α-stimulated (Fig. 3A, bar 6 versus bar 3), HK migration. Second, two designed RNAi sequences, RNAi-1 and RNAi-2, completely downregulated endogenous LRP1, as shown in Fig. 3B (insert, lane 2 and 3 versus lane 1). When these LRP1-null HKs were subjected to migration assays, either under normoxia plus HSP90α or hypoxia, they failed to exhibit increased migration in response to either hypoxia (Fig. 3B, bars 5 and 8) or HSP90α (Fig. 3B, bars 6 and 9). Third, to ensure the specificity of LRP1 function, we re-introduced a siRNA-resistant functional fragment of the LRP1 receptor, mLRP1 (Obermoeller-McCormick et al., 2001), into the LPR1-downregulated HKs. As shown in Fig. 3C, an expected major 150 kDa m-LRP1 receptor and a partially processed 85 kDa subunit were detected by anti-LRP1 antibody immunoblotting (against the p85 subunit of LRP1). The HKs expressing mLRP1 regained migratory responsiveness to both HSP90α and hypoxia (bars 8 and 9 versus bars 5 and 6). Taken together, these data provided evidence of a novel autocrine mechanism by which hypoxia promotes HK migration: hypoxia → HIF1 → HSP90α secretion → LRP1 receptor → cell migration. A schematic representation of these findings is shown in Fig. 3D.

LRP1 belongs to a family of seven members related to the LDL receptor (Lillis et al., 2008). Deletion of the LRP1 gene leads to

embryonic lethality in mice (Herz et al., 1992). It has been reported to bind a wide variety of extracellular molecules, including lipoproteins, proteases and their inhibitors, ECMs and growth factors (Lillis et al., 2008). Whereas LRP1 is widely expressed in different cell types, its expression is altered in breast cancer cells. In these cells, this alteration correlates with the invasiveness of the cancer (Lillis et al., 2008). How LRP1 mediates HSP90α signaling to promote HK migration remains largely unclear. A number of reports linked LRP1 signaling to the PDGF receptor. Activation of the PDGF receptor led to LRP1 tyrosine phosphorylation (Boucher et al., 2002; Loukinova et al., 2002). However, we observed that downregulation of LRP1 had little effect on TGFα-stimulated HK migration (Cheng et al., 2008) or PDGF-BB-stimulated dermal fibroblast migration (C.-F.C. and W.L., unpublished). Nonetheless, the main discovery of this work, that the HSP90α-LRP1 autocrine loop plays an essential role in hypoxia-driven cell migration, may have broad implications.

Materials and Methods

Primary human neonatal keratinocytes (HKs) were purchased from Clonetics (San Diego, CA) and cultured in EpiLife medium with added human keratinocyte growth supplements (HKGS), according to the manufacturer's instructions. HKs at passages four to five were used in all the experiments throughout this study. Native rat-tail type I collagen was from BD Biosciences (Bedford, MA). Colloidal gold (gold chloride, G4022) was purchased from Sigma (St Louis, MI). The cDNAs that encode HIF1α (WT), HIF1αCA5 (constitutively active) and HIF1αΔNBΔAB (dominant negative) were gifts from Gregg Semenza (Johns Hopkins University, Baltimore, MA). Anti-HIF1α antibody (#610958) and anti-HIF1β antibody (#611078) were from BD Transduction Laboratories (Lexington, KY). Anti-HSP90α antibody (SPA-840) for western analysis and anti-HSP90α neutralizing antibody (SPS-771) were from Stressgen (Victoria, BC, Canada). Recombinant HSP90α was prepared in our laboratory as previously described (Cheng et al., 2008). Anti-LRP1/CD91 neutralizing antibody was purchased from Progen Biotechnik (Heidelberg, Germany).

Hypoxia

Multi-chamber OxyCycler C42 from BioSpherix (Redfield, NY) was used as an oxygen content controller in this study. This equipment allows creation of a full-range oxygen content regulation from 0.1% to 99.9%, as well CO_2 control from 0.1% to 20.0%. All media used in hypoxia experiments were pre-incubated in the chambers with the designated oxygen content overnight.

Cell migration assays

An updated protocol for the colloidal gold cell motility assay, including data and statistical analysis, and a recently modified in vitro wound-healing assay (including procedures for pre-coating with ECMs, cell plating, scratching and quantification of migration data) were recently described by us (Li et al., 2004; Fan et al., 2006).

Sub-cloning, production of lentivirus stocks and infection

Wild-type or mutant HIF1α cDNAs were inserted into the lentivirus-derived vector pRRLsinhCMV at *Bam*HI-*Eco*RI sites, tested, and confirmed as previously described (Li et al., 2007).

FG-12 RNAi delivery system

Four potential sites were selected and synthesized using the RNAi Selection Program (Yuan et al., 2004). The effectiveness of synthetic double strand siRNA in downregulation of LRP1/CD91 was measured in 293 cells by transfection and the cell lysates blotted with a corresponding anti-LPR1 antibody. The two most effective RNAi sequences were cloned into the lentiviral RNAi delivery vector, FG-12, as previously described (Qin et al., 2003). The two selected RNAi sequences (sense) against human LRP1 were used for FG-12 cloning, as previously described (Cheng et al., 2008).

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References

Basu, S., Binder, R. J., Ramalingam, T. and Srivastava, P. K. (2001). CD91 is a common receptor for heat shock proteins gp96, hsp90, hsp70, and calreticulin. *Immunity* **14**, 303-313.

Boucher, P., Liu, P., Gotthardt, M., Hiesberger, T., Anderson, R. G. and Herz, J. (2002). Platelet-derived growth factor mediates tyrosine phosphorylation of the cytoplasmic domain of the low Density lipoprotein receptor-related protein in caveolae. *J. Biol. Chem.* **277**, 15507-15513.

Cheng, C. F., Fan, J., Fedesco, M., Guan, S., Li, Y., Bandyopadhyay, B., Bright, A. M., Yerushalmi, D., Liang, M., Chen, M. et al. (2008). Transforming growth factor alpha (TGFalpha)-stimulated secretion of HSP90alpha: using the receptor LRP-1/CD91 to promote human skin cell migration against a TGFbeta-rich environment during wound healing. *Mol. Cell. Biol.* **28**, 3344-3358.

Clayton, A., Turkes, A., Navabi, H. and Mason, M. D. (2005). Tabi Z: induction of heat shock proteins in B-cell exosomes. *J. Cell Sci.* **118**, 3631-3638.

Fan, J., Guan, S., Cheng, C. F., Cho, M., Fields, J. W., Chen, M., Denning, M. F., Woodley, D. T. and Li, W. (2006). PKCdelta clustering at the leading edge and mediating growth factor-enhanced, but not ECM-initiated, dermal fibroblast migration. *J. Invest. Dermatol.* **126**, 1233-1243.

Hegmans, J. P., Bard, M. P., Hemmes, A., Luider, T. M., Kleijmeer, M. J., Prins, J. B., Zitvogel, L., Burgers, S. A., Hoogsteden, H. C. and Lambrecht, B. N. (2004). Proteomic analysis of exosomes secreted by human mesothelioma cells. *Am. J. Pathol.* **164**, 1807-1815.

Herz, J., Clouthier, D. E. and Hammer, R. E. (1992). LDL receptor-related protein internalizes and degrades uPA-PAI-1 complexes and is essential for embryo implantation. *Cell* **71**, 411-421.

Hunt, T. K., Niinikoski, J. and Zederfeldt, B. (1972). Role of oxygen in repair processes. *Acta Chir. Scand.* **138**, 109-110.

Jiang, B. H., Rue, E., Wang, G. L., Roe, R. and Semenza, G. L. (1996). Dimerization, DNA binding, and transactivation properties of hypoxia-inducible factor 1. *J. Biol. Chem.* **271**, 17771-17778.

Kelly, B. D., Hackett, S. F., Hirota, K., Oshima, Y., Cai, Z., Berg-Dixon, S., Rowan, A., Yan, Z., Campochiaro, P. A. and Semenza, G. L. (2003). Cell type-specific regulation of angiogenic growth factor gene expression and induction of angiogenesis in nonischemic tissue by a constitutively active form of hypoxia-inducible factor 1. *Circ. Res.* **93**, 1074-1081.

Lerman, O. Z., Galiano, R. D., Armour, M., Levine, J. P. and Gurtner, G. C. (2003). Cellular dysfunction in the diabetic fibroblast: impairment in migration, vascular endothelial growth factor production, and response to hypoxia. *Am. J. Pathol.* **162**, 303-312.

Li, W., Henry, G., Fan, J., Bandyopadhyay, B., Pang, K., Garner, W., Chen, M. and Woodley, D. T. (2004). Signals that initiate, augment, and provide directionality for human keratinocyte motility. *J. Invest. Dermatol.* **123**, 622-633.

Li, W., Li, Y., Guan, S., Fan, J., Cheng, C. F., Bright, A. M., Chinn, C., Chen, M. and Woodley, D. T. (2007). Extracellular hsp90α: linking hypoxia to human skin cell migration and wound healing. *EMBO J.* **26**, 1226-1233.

Lillis, A. P., Van Duyn, L. B., Murphy-Ullrich, J. E. and Strickland, D. K. (2008). LDL receptor-related protein 1, unique tissue-specific functions revealed by selective gene knockout studies. *Physiol. Rev.* **88**, 887-918.

Loukinova, E., Ranganathan, S., Kuznetsov, S., Gorlatova, N., Migliorini, M. M., Loukinov, D., Ulery, P. G., Mikhailenko, I., Lawrence, D. A. and Strickland, D. K. (2002). Platelet-derived growth factor (PDGF)-induced tyrosine phosphorylation of the low density lipoprotein receptor-related protein (LRP). Evidence for integrated co-receptor function betwenn LRP and the PDGF. *J. Biol. Chem.* **277**, 15499-15506.

Mogford, J. E., Tawil, N., Chen, A., Gies, D., Xia, Y. and Mustoe, T. A. (2002). Effect of age and hypoxia on TGFbeta1 receptor expression and signal transduction in human dermal fibroblasts: impact on cell migration *J. Cell Physiol.* **190**, 259-265.

Obermoeller-McCormick, L. M., Li, Y., Osaka, H., FitzGerald, D. J., Schwartz, A. L. and Bu, G. (2001). Dissection of receptor folding and ligand-binding property with functional minireceptors of LDL receptor-related protein. *J. Cell Sci.* **114**, 899-908.

O'Toole, E. A., Marinkovich, M. P., Peavey, C. L., Amieva, M. R., Furthmayr, H., Mustoe, T. A. and Woodley, D. T. (1997). Hypoxia increases human keratinocyte motility on connective tissue *J. Clin. Invest.* **100**, 2881-2891.

Qin, X. F., An, D. S., Chen, I. S. and Baltimore D. (2003). Inhibiting HIV-1 infection in human T cells by lentiviral-mediated delivery of small interfering RNA against CCR5. *Proc. Natl. Acad. Sci. USA* **100**, 183-188.

Semenza, G. L. (2000). HIF-1: mediator of physiological and pathophysiological responses to hypoxia. *J. Appl. Physiol.* **88**, 1474-1480.

Semenza, G. L. (2003). Targeting HIF-1 for cancer therapy. *Nat. Rev. Cancer* **3**, 721-732.

Strickland, D. K., Ashcom, J. D., Williams, S., Burgess, W. H., Migliorini, M. and Argraves, W. S. (1990). Sequence identity between the alpha 2-macroglobulin receptor and low density lipoprotein receptor-related protein suggests that this molecule is a multifunctional receptor. *J. Biol. Chem.* **265**, 17401-17404.

Tandara, A. A. and Mustoe, T. A. (2004). Oxygen in wound healing-more than a nutrient. *World J. Surg.* **28**, 294-300.

Woodley, D. T., Bachmann, P. M. and O'Keefe, E. J. (1988). Laminin inhibits human keratinocyte migration. *J. Cell Physiol.* **136**, 140-146.

Xia, Y. P., Zhao, Y., Tyrone, J. W., Chen, A. and Mustoe, T. A. (2001). Differential activation of migration by hypoxia in keratinocytes isolated from donors of increasing age: implication for chronic wounds in the elderly. *J. Invest. Dermatol.* **116**, 50-56.

Yu, X., Harris, S. L. and Levine, A. J. (2006). The regulation of exosome secretion: a novel function of the p53 protein. *Cancer Res.* **66**, 4795-4801.

Yuan, B., Latek, R., Hossbach, M., Tuschi, T. and Lewitter, F. (2004). siRNA Selection Server: an automated siRNA oligonucleotide prediction server. *Nucleic Acids Res.* **32**, W130-W134.