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論文題目	Crucial role of p63RhoGEF in chemotactic migration of breast carcinoma cells （ヒト乳癌細胞の細胞遊走における p63RhoGEF の役割）
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論文内容の要旨

Cell migration plays an essential role in physiological and pathological events, including embryonic development, immune responses, tissue maintenance, and tumor metastasis. Migration is mediated by reorganization of actin cytoskeleton. When exposed to chemotactic factors, cells become polarized and form an F-actin-rich lamellipodial membrane protrusion toward the direction of cell movement. Rho family GTPases, such as RhoA, Rac1 and Cdc42, are key regulator in actin cytoskeletal reorganization, and spatiotemporally control actin dynamics during cell migration. Rho GTPases act as a molecular switch that shifts between an active GTP-bound form and an inactive GDP-bound form. Rho GTPases are activated by Rho guanine nucleotide-exchange factors (Rho-GEFs), which catalyze the exchange of GDP for GTP in Rho-GTPases. In the human genome, there are approximately 70 Dbl-like Rho-GEF genes. Existence of the large number of Rho-GEFs, compared with number (approximately 20) of Rho family GTPases, raises the possibility that each Rho-GEF selectively regulates specific functions of its target Rho family GTPases. Thus, it is important to determine the functional roles and regulation mechanisms of each Rho-GEF in the context of various cellular processes.

To identify Rho-GEFs that involved in serum-induced migration in tumor cells, Rho-GEF shRNAs were screened for suppression of serum-induced chemotactic migration in MDA-MB-231 human breast carcinoma cells. Of shRNAs targeting 66 Rho-GEFs, I identified that at least the six Rho-GEFs (AAH33666, Duet, Frabin/FGD4, Net1, p63RhoGEF and Trio) are involved in serum-induced chemotactic migration of MDA-MB-231 cells. In this study, I focused on the role of p63RhoGEF, which specifically activate RhoA, in cell migration. Although p63RhoGEF has been studied its role in contraction of vesicular smooth muscle cells, it is unclear about its functional role in cell migration. I examined the effects of knockdown of p63RhoGEF on serum-induced RhoA activation. Knockdown of p63RhoGEF significantly suppressed the serum-induced RhoA activation. This result indicates that p63RhoGEF is crucial for serum-induced RhoA activation in MDA-MB-231 cells. I also examined the activity of p63RhoGEF. The level of active p63RhoGEF gradually increased and reached a maximum at 20 min and then maintained after serum stimulation, as measured by pull-down assay using GST-RhoA(G17A). These results indicate that p63RhoGEF is activated by serum stimulation and is involved in serum-induced RhoA activation in MDA-MB-231 cells. I then measured the effect of p63RhoGEF expression of the actin reorganization of MDA-MB-231 cells. Consistent with its RhoA-specific activity, expression of p63RhoGEF induced cell rounding and the formation of stress

fibers and suppressed the formation of F-actin-rich lamellipodia. In order to further investigate the role of p63RhoGEF in serum-induced cell migration, I analyzed the effect on p63RhoGEF on directional (chemotactic) and random (chemokinetic) migration of MDA-MB-231 cells. As already noted, knockdown of p63RhoGEF significantly suppressed serum-induced chemotactic cell migration, but had no significant effect on the chemokinetic cell migration. This result suggested that p63RhoGEF plays a more specific role in chemotactic migration than chemokinetic migration in MDA-MB-231 cells. To elucidate the role of p63RhoGEF in cell migration, the effect of knockdown of p63RhoGEF on actin cytoskeleton and cell morphology were analyzed. Knockdown of p63RhoGEF had no apparent effect on the overall cell shape and F-actin organization before serum stimulation, but caused the production of multiple lamellipodial protrusion around the cell periphery after serum stimulation. Similar results were obtained by overexpression of p63RhoGEF(L301E), a GEF-inactive mutant of p63RhoGEF. Taken together, these results suggest that p63RhoGEF plays a crucial role in serum-induced chemotactic migration by facilitating the formation of the single polarized lamellipodial protrusion in response to serum stimulation.

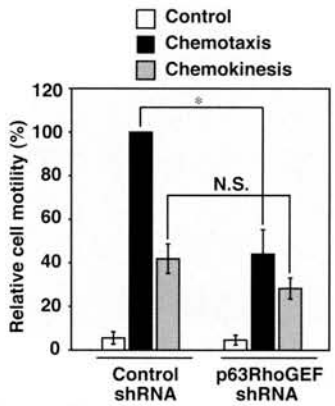

Figure 1. Knockdown of p63RhoGEF suppresses chemotactic migration, but not chemokinetic migration

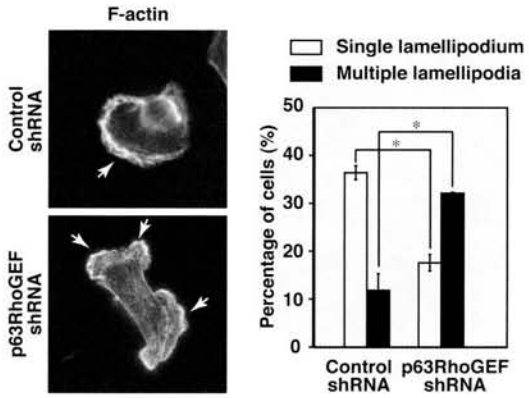

Figure 2. p63RhoGEF is required for the formation of a single polarized lamellipodium.

論文審査結果の要旨

細胞遊走は、胚発生、免疫応答、癌浸潤・転移など多くの生理的、病理的現象に関与している。細胞遊走において、Rho ファミリーGTPase によるアクチン細胞骨格の時空間的な制御機構が重要な役割を担っている。Rho ファミリーは Rho-guanine nucleotide-exchange factor（Rho-GEF）によって活性化されるが、細胞遊走に関わる Rho-GEF の実体は不明であった。本研究では、ヒト乳癌細胞 MDA-MB-231 細胞の血清刺激依存的な細胞遊走に関与する Rho-GEF を同定し、機構解析を行った。66 種類の Rho-GEF に対する shRNA を用いてスクリーニングを行った結果、6 種類の Rho-GEF（AAH33666、Duet、Frabin/FGD4、Net1、p63RhoGEF、Trio）を発現抑制すると、細胞遊走が有意に抑制された。この中でも、RhoA 特異的な Rho-GEF である p63RhoGEF に注目し、細胞遊走における機能を解析した。p63RhoGEF の発現抑制や GEF 不活性型変異体の過剰発現によって、血清刺激依存的な RhoA の活性化が抑制された。また、血清刺激によって p63RhoGEF が活性化した。これらの結果から、血清刺激によって p63RhoGEF が活性化し、血清刺激依存的な RhoA の活性化において重要な役割を担っていることが示された。また、p63RhoGEF の発現抑制によって、方向性をもった細胞遊走（ケモタキシス）が抑制されたが、方向性のない運動性（ケモキネシス）には影響を与えなかったことから、p63RhoGEF はケモタキシスに関与することが示唆された。さらに、p63RhoGEF の発現抑制や GEF 不活性型変異体の過剰発現によって、多方向に複数のラメリポディアを形成する細胞が有意に増加した。これらの結果から、p63RhoGEF は、血清刺激による RhoA の活性化を介して、一方向にラメリポディアを形成することで、方向性を持った細胞遊走に関与していることが示された。以上の成果は、細胞遊走の分子機構を理解し、癌細胞の浸潤、転移機構を解明する上で重要な成果であり、本論文は、著者が自立して研究活動を行うに必要な高度の研究能力と学識を有することを示している。したがって、林文提出の論文は、博士（生命科学）の博士論文として合格と認める。