Review

Possible Therapeutic Applications of Targeting STAP Proteins in Cancer

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The signal-transducing adaptor protein (STAP) family, including STAP-1 and STAP-2, contributes to a variety of intracellular signaling pathways. The proteins in this family contain typical structures for adaptor proteins, such as Pleckstrin homology in the N-terminal regions and SRC homology 2 domains in the central regions. STAP proteins bind to inhibitor of kappaB kinase complex, breast tumor kinase, signal transducer and activator of transcription 3 (STAT3), and STAT5, during tumorigenesis and inflammatory/immune responses. STAP proteins positively or negatively regulate critical steps in intracellular signaling pathways through individually unique mechanisms. This article reviews the roles of the novel STAP family and the possible therapeutic applications of targeting STAP proteins in cancer.

Key words signal-transducing adaptor protein (STAP); adaptor protein; signal transduction; cancer cell growth; tumorigenesis

1. INTRODUCTION

At the present time, cancer is a major public health problem worldwide. New findings for specific molecules in different types of cancers will provide the development of new types of drugs that can inhibit oncogenic signals. Aberrant activation of intracellular signals, mediated by the oncogenic properties of kinases, is a cause of carcinogenesis.[1–3] Meanwhile, adaptor proteins exert positive or negative regulatory functions by targeting components of the kinase signaling cascade.[4] Members of the signal-transducing adaptor protein (STAP) family contribute to various signaling events for cancer cell growth and immune responses. The STAP family contains STAP-1 and STAP-2. STAP-1 is also known as B-cell antigen receptor downstream signaling 1 (BRDG1), which was identified as a protein phosphorylated by Tec tyrosine kinase.[5] With the yeast two-hybrid screening of a hematopoietic stem cell library, STAP-1 was further isolated as a c-KIT-interacting protein.[6] STAP-2 was identified as a c-FMS-binding partner.[7] Both STAP-1 and STAP-2 (overall amino acid identity, 33%) contain a Pleckstrin homology (PH) domain in their N-terminal region and a region weakly related to a Src homology 2 (SH2) domain in their central region (Fig. 1). The amino acid sequences of the PH domains have 36% identity and 58% similarity between STAP-1 and STAP-2. The amino acid sequence identity of the SH2 domain of STAP-2 is 40% with that of STAP-1 and 29% with the SH2 domain of human phospholipase C-γ2. In the C-terminal region, STAP-2, but not STAP-1, carries a proline-rich region with a signal transducer and activator of transcription 3 (STAT3)-binding YXXQ motif.

Expression of STAP-1 is relatively restricted to hematopoietic cells,[5,6] but its expression is promoted in pro-inflammatory microglia and macrophages that are involved in neuronal apoptosis and degeneration.[8] STAP-1 mutations have been identified in some patients with autosomal dominant hypercholesterolemia,[9,10] but functional meanings of STAP-1 in cholesterol homeostasis remain controversial.[11,12] STAP-1 functions to regulate maintenance and activation of invariant NKT cells, and contributes to the pathogenesis of autoimmune hepatitis.[13] A recent study demonstrated a critical role of STAP-1 in the maintenance of chronic myeloid leukemia (CML) leukemia stem cells (LSCs).[14]

STAP-2 is expressed in various types of cells and tissues, including lymphocytes, macrophages, dendritic cells, and hepatocytes.[7] The abundant expression pattern indicates that STAP-2 widely contributes to various signaling and transcriptional molecules (Fig. 1). In T cells, STAP-2 regulates STAT5-mediated expression of cytokine-responsible genes and enhances activation of the Fas-induced caspase cascade.[7] In macrophages and dendritic cells, STAP-2 upregulates FcεRI- and Toll-like receptor-mediated signals.[7] Therefore, STAP-2 can regulate both immune and inflammatory systems. As shown in Fig. 2, STAP-2 associates with breast tumor kinase (BRK) and STAT3, resulted in increased growth capacity of T47D breast cancer cells *via* enhanced BRK-mediated STAT3 activation.[15] In B16F10 melanoma cells, STAP-2 upregulates tyrosinase protein content, which determines tumor invasion *via* controlling expression of chemokine receptors.[16] In CML cells, STAP-2 interacts with a fusion oncoprotein BCR-ABL resulted in obvious increase of its downstream signals.[17] For these effects, the PH and SH2 domains have an ability to bind to several signaling molecules as shown in Fig. 3. Thus, STAP proteins contribute to the onset and progression of several types of cancers as described below.

2. STAP PROTEINS IN CANCER

2.1. STAP-2 in Breast Cancers Breast cancers are the most frequent cause of cancer-related deaths in women. The 5-year survival rate of breast cancer is relatively high, but undesirable recurrences and fatalities still remain to be solved. Its prognosis is predicted from clinicopathological classification determined by tumor subtype, histological grade, tumor

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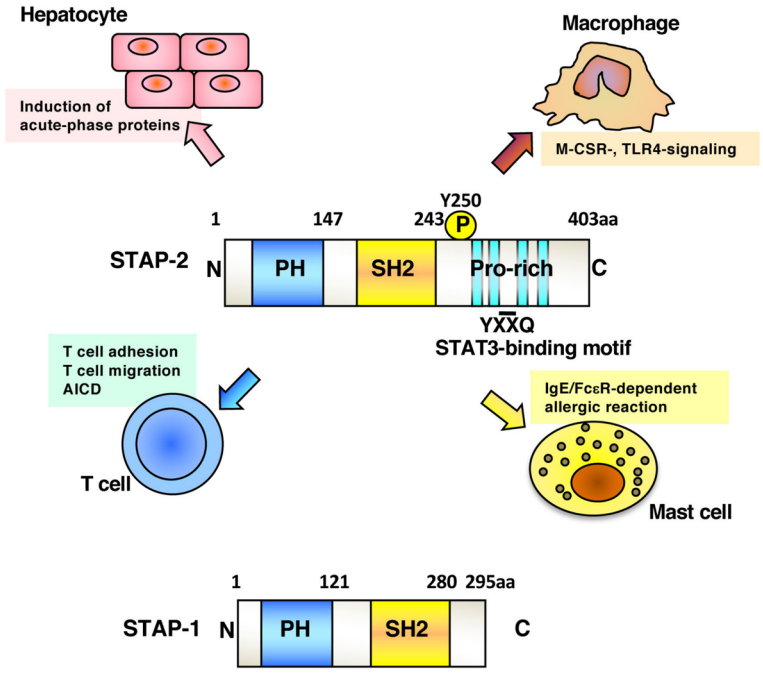

Fig. 1. Structural Features of STAP-1 and STAP-2, and STAP-2 Functions in Various Intracellular Signalings in Several Cell Types

Both contain an amino (N) terminal pleckstrin homology (PH) domain, a central SRC homology 2 (SH2) domain, and STAP-2 has an additional carboxy (C)-terminal proline-rich domain. STAP-2 is phosphorylated at Tyr 250 (Y250) by a variety of protein tyrosine kinases. The YXXQ motif is involved in the STAT3-mediated signaling. aa: amino acid. M-CSF: macrophage-colony stimulating factor; TLR4: toll-like receptor 4; AICD: activation induced cell death; FcεR: Fc epsilon receptor. (Color figure can be accessed in the online version.)

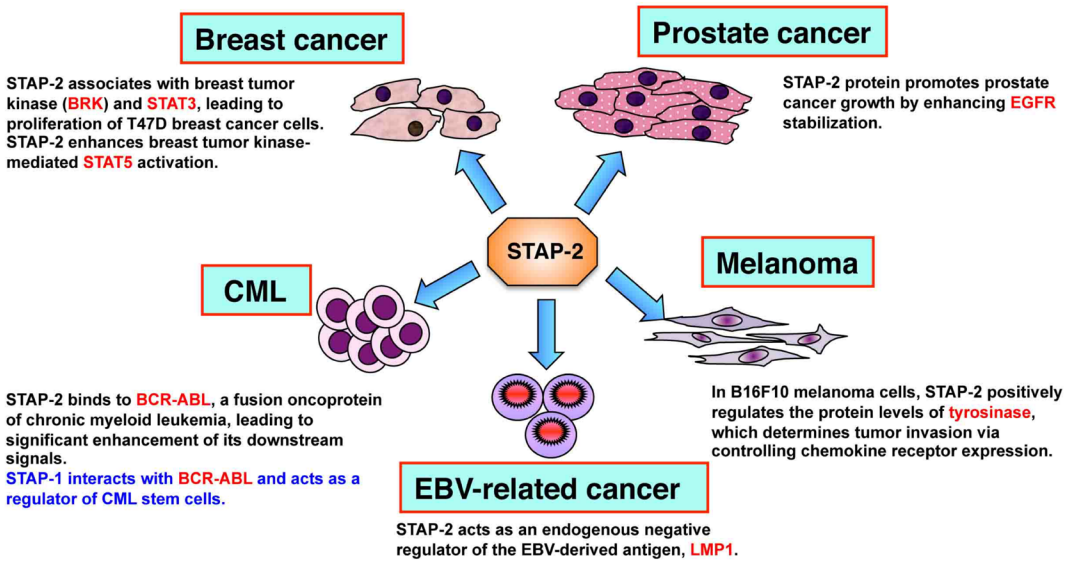

Fig. 2. STAP-2 Regulates a Variety of Intracellular Signalings in Several Types of Cancer Cells

EGFR: epidermal growth factor receptor; CML: chronic myelogenous leukemia; EBV: Epstein–Barr virus; LMP1: latent membrane protein 1. (Color figure can be accessed in the online version.)

size, and nodal status.[18] Although several molecular markers have been indicated as valuable prognostic factors in breast cancers, the expression pattern of estrogen receptor (ER), progesterone receptor as well as human epidermal growth factor receptor 2 (HER2) is currently utilized for subtyping.[19] Hormone-sensitive luminal early breast cancers show low recurrence rates after surgery. HER2-positive breast cancers respond well to the treatment with anti-HER2 antibodies, including Herceptin (also known as Trastuzumab). In contrast, triple-negative (TN) breast cancers with neither hormone receptors nor HER2 amplification show high recurrence rates with metastases, despite aggressive treatment with chemotherapy and/or radiotherapy.[19] Thus, new therapeutic strategies are particularly required for TN breast cancers. Recently, novel targets, such as vascular endothelial growth factor receptor, epidermal growth factor receptor (EGFR), mammalian target of rapamycin, poly-(ADPribose)-polymerase 1, SRC tyrosine kinase, BRK, breast cancer susceptibility genes (BRCA), and heat shock protein 90, are reported to have therapeutic potential for these types of breast cancers.[20,21]

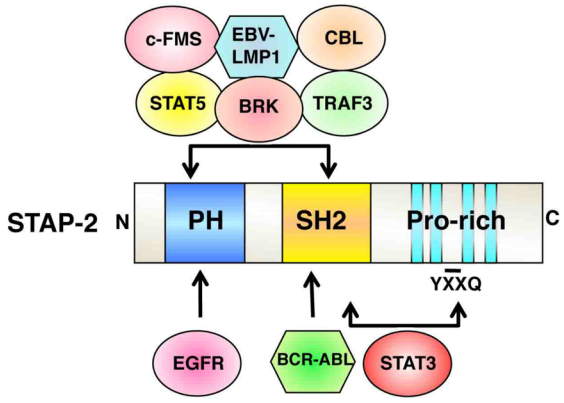

Fig. 3. Molecular Interactions between STAP-2 and Several Signaling Molecules

cFMS: macrophage-colony stimulating factor; EBV-LMP1: Epstein–Barr virus latent membrane protein 1; CBL: casitas B-lineage lymphoma; TRAF: tumor necrosis factor receptor-associated factor; PH: pleckstrin homology; SH2: src homology 2. (Color figure can be accessed in the online version.)

BRK is overexpressed in about 85% of invasive ductal breast cancers.[20] Expression, activation, and amplification of the BRK gene have been reported in HER2-positive mammary gland cancers.[22] BRK is distantly related to SRC family tyrosine kinases, and its activity is suppressed by phosphorylation of its C-terminal tyrosine residue.[20] However, BRK is not myristoylated; therefore, BRK usually localizes in the nucleus, where it has unique sets of substrates and interacting proteins. STAP-2 was the first substrate identified for BRK.[7] STAP-2 binds to BRK *via* its PH domain, and is involved in induction of robust activation of STAT3[15] (Fig. 4A). The binding potential of STAP-2 PH domain to BRK is likely to be powerful information because the PH domain is essential for STAP-2 translocation into the membrane after EGF-stimulation.[7] Therefore, STAP-2 PH domain may have an ability to alter intracellular localization of BRK for its elevated activation. STAT3 activation by BRK is also a crucial event for breast cancer T47D cell growth.[15] In this process, STAP-2 acts as a scaffold protein to enhance interactions between BRK and STAT3. Taken together with experiments using deletion mutants, STAP-2 contributes to multiple events, such as binding of STAP-2 to BRK and elevated activation of BRK, followed by enhancement of STAT3 tyrosine phosphorylation. Thus, STAP-2 cooperates with BRK, leading to enhancement of breast cancer cell growth.

BRK also upregulates tumor invasion *via* inducing phosphorylation of focal adhesion protein paxillin, resulted in activation of the small guanosine 5′-triphosphatase (GTPase) RAC1 through CRKII function.[20] In addition, BRK regulates both the small GTPase RHOA and RAS through p190RHO GAP-A phosphorylation during malignant transformation of mammary cells.[20] These findings are interesting because STAP-2 is known to bind to VAV1, a guanine-nucleotide exchange factor for RAC1, leading to activation of RAC1 signaling during SDF-1α-induced T cell chemotaxis.[7]

Because both BRK and STAP-2 are highly expressed in breast cancer cells, their linkage may promote dysregulated STAT3 activation. The above data clearly propose underlying molecular mechanisms and meanings of the BRK/STAP-2/STAT3 interactions, and may provide insights to develop new therapeutic strategies for breast cancers.

STAP-2 also interacts with STAT5 in breast cancer cells.[23] Two STAT5 isoforms, STAT5a and STAT5b, closely links on human chromosome 17,[23] and have approximately 96% sequence similarity. STAT5a was cloned as a mammary gland factor to enhance milk protein production, while STAT5b was isolated as a signaling molecule to mediate growth hormone-related function in the mammary gland. In addition, STAT5 activation is induced by a variety of cytokines. Although STAP-2 down-regulates STAT5 activation in erythropoietin-, interleukin-2 (IL-2)-, and IL-3-induced signaling,[7] its expression augments BRK-mediated STAT5 activation in breast cancer cells. Although explanation of different effects of STAP-2 on STAT5 activation is difficult, some stimulatory signal- or cell type-specific factors may determine STAP-2 functions. Importantly, both STAT5a and STAT5b are highly expressed and/or constitutively activated in a variety of malignancies, including breast cancers.[23,24] Recent evidence suggests that STAT5b has pro-proliferative roles in breast cancers, head and neck cancers, and prostate cancers, while STAT5a does not.[25]

Because BRK and STAP-2 synergistically activate STAT3 and STAT5, evaluation of expression of BRK combined with STAP-2 is likely to establish more meaningful prognostic scores for breast cancers than BRK expression alone. Furthermore, STAP-2 is a possible therapeutic target in BRK-expressing breast cancers. Although we do not know whether the other treatments involve STAP-2-mediated signal transduction, inhibitors of STAP-2 function are likely to have potential for development as anticancer drugs for breast cancers.

2.2. STAP-2 in Prostate Cancer Most of prostate cancers well respond to androgen-deprivation therapy because their tumor growth is initially dependent on androgens.[26] However, castration-resistant prostate cancers develop resistance to anti-androgen therapies, and are a major cause of death. Molecular mechanisms underlying prostate cancer initiation and progression include persistent androgen receptor activity, phosphatase and tensin homolog deleted from chromosome 10 (PTEN) deletion, and activation of phosphatidylinositol 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) signaling as well as EGFR and intracellular kinases.[26] In particular, constitutive EGFR activation is a possible mechanism to drive malignant transformation, and EGFR inhibitors are clinically utilized to treat patients with some types of malignancies, including lung cancers.[27] However, phase II trials of an EGFR inhibitor, Gefitinib, for patients with prostate cancers reveal only limited efficacy,[28] suggesting that prostate cancer cells may have an unknown mechanism to enhance the EGFR signaling pathway that defects in lung cancer cells. Thus, new treatment strategy based on understanding of more detailed mechanisms for EGFR activation in prostate cancer cells seems to be required.

STAP-2 augments EGFR-mediated signals through its protein stabilization, leading to high tumor formation of DU145 prostate cancer cells[29] (Fig. 4B). STAP-2 enhances EGFR signaling by two steps: EGFR stabilization and STAT3 upregulation *via* their direct association. In addition of EGFR-signaling, STAT3 is also activated by IL-6 receptor-signaling, and blockade of IL-6 receptors significantly suppresses tumor growth.[30] Because STAP-2 contributes to both pathways, STAP-2 knockdown can repress prostate cancer cell growth through synergistic inhibition of EGFR- and IL-6 receptor-signaling. As mentioned in the section of breast cancers,

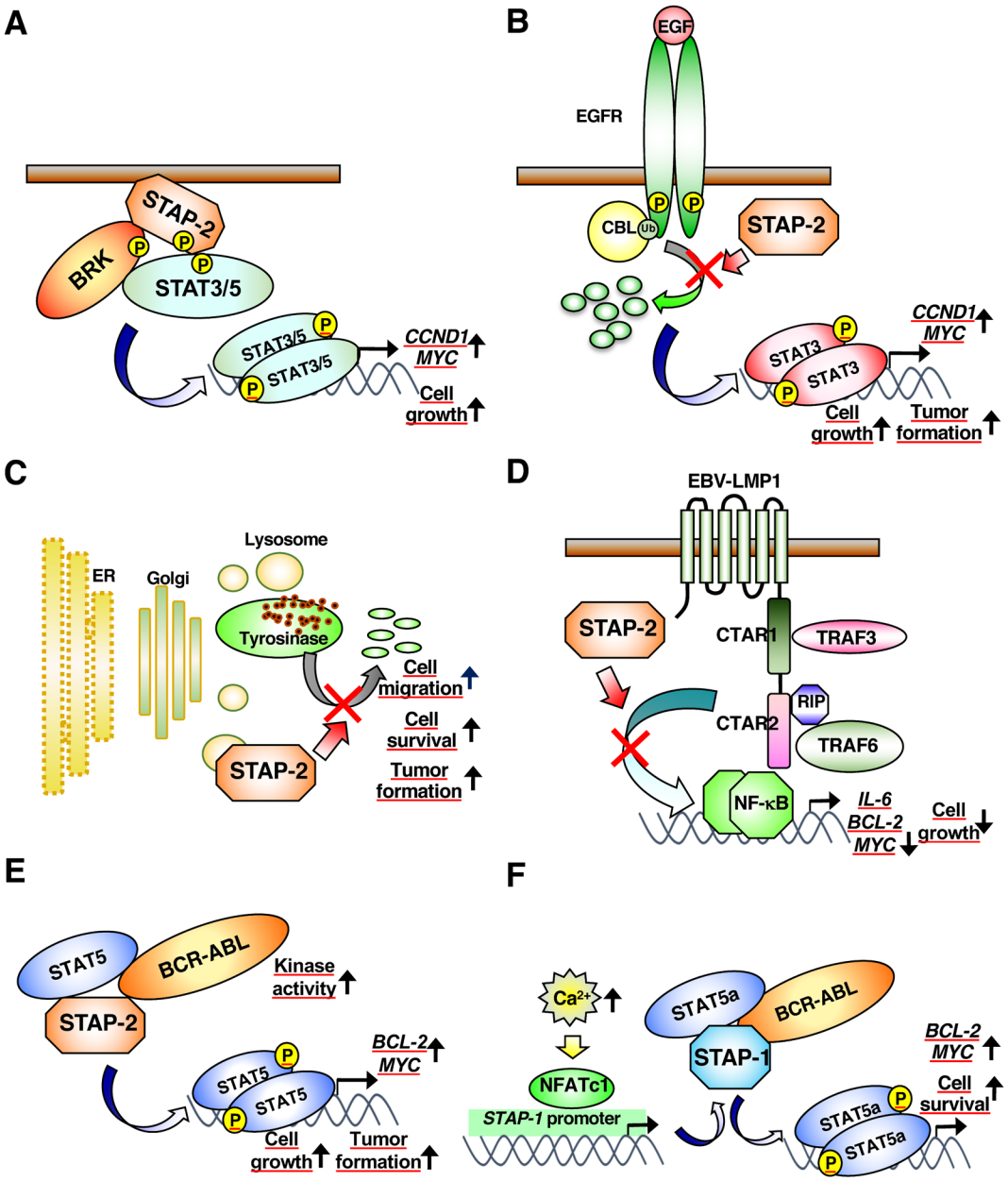

Fig. 4. Molecular Mechanisms Underlying the STAP's Functions in Several Types of Cancer Cells

A, In breast cancer cells, STAP-2 can enhance the Brk-mediated STAT3/5 activation. B, In prostate cancer cells, STAP-2 can enhance the EGFR-mediated signaling by EGFR protein stabilization and STAT3 upregulation. C, In melanoma cells, STAP-2 can modify cancer phenotype *via* the protection of tyrosinase against lysosomal degradation. D, In EBV-transformed cancer cells, STAP-2 can act as an endogenous negative regulator of the EBV-derived antigen LMP1. RIP: receptor-interacting protein. E, In CML cancer cells, STAP-2 can modulate the BCR-ABL-mediated cell growth and tumorigenesis. F, In CML cancer cells, STAP-1 can enhance BCR-ABL-induced STAT5 activation that affects cell cycle, anti-apoptotic mechanism. In addition, the Ca^{2+}/NFAT signals can induce STAP-1 mRNA expression. NFAT; nuclear factor of activated T-cells. (Color figure can be accessed in the online version.)

STAP-2 has an ability to bind to BRK and to enhance BRK-mediated activation of STAT3 and STAT5.[15,23] Of note, BRK upregulates EGFR-signaling through suppressing casitas B-lineage lymphoma (CBL)-promoted ubiquitination of EGFR.[31] EGFR dimerizes upon ligand ligation, and subsequently associates with GRB2, followed by RAS activation. Activated RAS then promotes activation of extracellular signal-regulated kinase (ERK) that induces cancer cell proliferation. Cell surface expression of EGFR is critical to induce RAS and ERK activation, and STAP-2 expression suppresses decrease of surface EGFR protein even after EGF-stimulation. Another functional mechanism of STAP-2 in prostate cancer cells is inhibition of CBL-promoted EGFR ubiquitination, resulted in EGFR restoration.[29] EGFR forms homodimers or heterodimers with HER2, HER3, or HER4 after the binding of EGF to the receptors. The dimerized EGFR then activates downstream signaling molecules, such as AKT and ERK. CBL-promoted ubiquitination of EGFR heterodimers occurs slower than that of EGFR homodimers; therefore, overactivation of EGFR signaling and elevated growth capacity are frequently observed in HER2- or HER3-overexpressing cancer cells.[32] STAP-2 knockdown down-regulates cell growth of prostate cancer DU145 cells and LNCaP cells.[29] STAP-2 overexpression restores surface expression of EGFR.[29] STAP-2 fails to associate with a dimerization-deficient mutant EGFR K721A; therefore, STAP-2 seems to interact with EGFR after its

dimerization process.[29] In addition, STAP-2 stabilizes wild-type EGFR, but not an inactive EGFR mutant, after EGF-stimulation.[29] Indeed, STAP-2 knockdown-induced repression of tumor cell growth is observed under EGFR-activated, but not under EGFR-inactivated, conditions.[29] Importantly, Gefinitib treatment fails to further inhibit cell growth of STAP-2-knockdown prostate cancer DU145 cells.[29] Different regulatory mechanisms for EGFR surface expression between cells treated with Gefinitib and STAP-2 knockdown may suggest that STAP-2 inhibition could destabilize both wild-type EGFR and Gefitinib-resistant autoactivated EGFR.[29] Therefore, STAP-2 inhibitors are likely to have potential for development as anticancer drugs for Gefitinib-resistant prostate cancers.

2.3. STAP-2 in Melanoma Melanoma arises from genetic mutations in melanocytes of the skin, eye, inner ear, and leptomeninges, and progresses quickly and undergoes tumorigenic evolution.[33,34] Although melanoma patients receive combined therapies of surgery, radiation, chemotherapy, and immunotherapy, most of patients with distant metastases will die within 5 years.[33,34] Thus, new therapeutic approaches are really required. Of note, a mutation in exon 15 of *BRAF* is reported in 50–70% of melanoma cases.[33,34] From results of recent clinical trials, inhibition of the BRAF–MAP kinase pathway is now expected to prolong progression-free and overall survival.[35] However, melanoma is still unique in its high ability for melanogenesis and metastasis.

A murine B16F10 cell line was originally established from a spontaneously occurring melanoma in C57BL/6 mice and selected on the basis of its high lung colonization capacity.[36] Notably, B16F10 cells with manipulation of STAP-2 expression show different characteristics in cell shape, melanin production, cell growth, and chemokine receptor expression as compared with original cells. In addition, STAP-2-deficient B16F10 cells forms tumors in completely different pattern of organs when injected to mice.[16]

To achieve these changes, STAP-2 is thought to protect tyrosinase from lysosomal degradation. Tyrosinase, whose gene is located in the *albino* locus, is a rate-limiting enzyme to catalyze tyrosine hydroxylation during melanogenesis.[37] Mutations of *tyrosinase* gene in humans cause an inherited oculocutaneous albinism, characterized by the absent of pigmentation of skin, hair, and eyes.[38] In B16F10 cells, STAP-2 dose-dependently prevents tyrosinase from protein degradation (Fig. 4C). Indeed, STAP-2 knockdown significantly decreased tyrosinase protein level, while B16F10 cells overexpressing STAP-2 exhibit increased tyrosinase content.

Ascorbic acid has an ability to suppress tyrosinase activity and melanin formation, and can promote caspase-8-independent apoptosis of B16F10 cells.[39] These findings seem to indicate the involvement of tyrosinase in melanogenesis and survival of B16F10 cells. Furthermore, main colonization organs of mice injected with B16F10 cells are changed from the lung to the liver by knockdown of either STAP-2 or tyrosinase expression.[16] Thus, tyrosinase, whose protein levels are partly controlled by STAP-2, determines tumor invasion by regulating chemokine receptor expression.

Melanoma cells express various chemokine receptors, such as CCR7, CCR10, and CXCR4. CCR7 and CCR10 expression is connected with rapid progression and poor prognosis.[40] CCR7-overexpressing B16F10 cells severely infiltrate into the draining lymph nodes,[41] while overexpression of CXCR4 promotes massive metastasis to the lung.[42] In addition, decrease of CXCR3 expression promotes impaired capacity of metastasis to the lymph nodes.[43] Notably, protein content of STAP-2 or tyrosinase greatly influences expression of chemokine receptor on B16F10 cells.[16] Knocked down of STAP-2 or tyrosinase induces decreased CXCR4 expression and increased CXCR3 expression.[16] Thus, STAP-2 and tyrosinase affect expression pattern of chemokine receptors; therefore, they partly determine organs of B16F10 cell-colonization *in vivo*.[16]

Regulation of tyrosinase protein content by STAP-2 occurs at the post-transcriptional level.[16] Tyrosinase protein is proteolyzed through ER-associated protein degradation, as well as being degraded after its complete maturation in the Golgi.[16] In addition, the tyrosinase protein content is regulated by the ubiquitin–proteasomal pathway, and tyrosinase also is degraded in the lysosomes.[16] Both STAP-2 and tyrosinase co-exist in the lysosomes, as well as in the ER and Golgi.[16] Of note, decrease of tyrosinase protein content observed in shSTAP-2 cells is clearly restored in the presence of NH_4Cl.[16] Thus, STAP-2 colocalizes with tyrosinase in the place where tyrosinase processing occurs in melanocytes. However, STAP-2 failed to associate with tyrosinase, suggesting that its influence on tyrosinase may be indirect.[16] Alternatively, STAP-2 expression may regulate the condition and/or activity of lysosomes.

Taken together, STAP-2 contributes to melanogenesis, and its expression regulates metastatic phenotypes of melanoma. STAP-2 represents a suitable molecular target for the establishment of novel therapeutic approaches toward melanoma, and evaluation of STAP-2 expression has a potential to provide meaningful information into unique characteristics of melanoma.

2.4. STAP-2 in Epstein–Barr Virus (EBV)-Related Malignancy EBV transforms resting B cells into proliferating lymphoblastoid cells through promoting constitutively activated nuclear factor-kappaB (NF-κB) by oncogenic protein latent membrane protein 1 (LMP1), a product of EBV.[44] Consequently, LMP1 is involved in the pathogenesis of EBV-related human lymphoma and nasopharyngeal carcinoma. LMP1 is a transmembrane protein that activates NF-κB through two C-terminal activation regions (CTARs), CTAR1 and CTAR2, that hijack signaling and adaptor proteins, including tumor necrosis factor (TNF)-receptor-associated factors (TRAFs), receptor-interacting protein 1 (RIP1), and TNFRSF1A-associated *via* death domain.[44] Both CTARs are essential for induction of B-cell transformation. CTAR2 selectively promotes activation of the canonical NF-κB pathway, while CTAR1 activates the noncanonical NF-κB pathway, which regulates NF-κB2/p100 precursor processing.

STAP-2 inhibits LMP1-induced IL-6 expression, which promotes EBV-transformed B-cell growth through both canonical and non-canonical NF-κB activation[45] (Fig. 4D). STAP-2 directly interacts with the C-terminal domain of LMP1 through its PH and SH2 domains in EBV-positive human B cells. With regard to the mechanism, STAP-2 enhances binding of LMP1 to TRAF3. STAP-2 can recognize both LMP1 and TRAF3, and has a similar intracellular distribution to LMP1, suggesting that it functions to bridge LMP1 and TRAF3 *in vivo*. TRAFs are essential adaptor proteins underlying in the downstream of many receptors, such as TNFR and IL-1 receptor/Toll-like receptor superfamilies. While TRAF2, TRAF5, and

TRAF6 activate the canonical NF-κB pathway, TRAF3 inhibits the non-canonical NF-κB pathway.[44] Indeed, reduction of endogenous STAT2 or TRAF3 enhances LMP1-induced NF-κB activation.[45] More interestingly, STAP-2 mRNA is induced by LMP1 expression, and transient STAP-2 expression suppresses cell growth of EBV-positive human B cells.[45] Furthermore, STAP-2-deficient murine embryonic fibroblasts show enhanced LMP1-induced cell growth.[45] Therefore, STAP-2 acts as an endogenous negative regulator of EBV-derived antigen LMP1.

NF-κB activation is required for lymphocyte proliferation, differentiation, and survival through induction of genes relating to lymphocyte biology, such as Cyclin D1, c-Myc, Bcl-2 family members as well as immune/inflammation regulatory cytokines.[46] Because NF-κB simultaneously provides signals to promote cell growth and survival, constitutive NF-κB activation may cause development of malignant lymphomas. Indeed, aberrant activation of NF-κB is reported in a variety of lymphoid malignancies.[47] In addition to EBV, there are two types of human lymphomagenic viruses, which encodes NF-κB-activating oncoproteins. Kaposi's sarcoma-associated herpes virus, which is related to primary effusion lymphoma, produces a homolog of cellular FLICE-like inhibitory protein to activate the NF-κB pathway.[48] Human T-lymphotropic virus type I, which is a main cause for adult T-cell lymphoma/leukemia, produces 40-kDa oncoprotein Tax to immortalize primary human T cells as well as to transform rodent fibroblasts.[49] Tax transgenic mice are reported to develop leukemia and lymphomas.[50] Of note, STAP-2 expression greatly inhibits LMP1-induced NF-κB activation, suggesting that STAP-2 may act to limit EBV infection in lymphocytes.[45] In addition, STAP-2 expression is induced by LMP1; therefore, human beings have gained this control system involving STAP-2 as a defense against EBV infection.[45] Interestingly, recovery of B lymphocytes following transplantation is augmented in the absence of STAP-2.[51] Furthermore, specific overexpression of STAP-2 in lymphoid cells reduced the numbers of late-stage B lymphocyte progenitors within the bone marrow.[51] Therefore, STAP-2 has suppressive effects in normal and malignant B lymphocyte-specific manners.

2.5. STAP-1 and STAP-2 in CML CML is a clonal myeloproliferative disease whose initial chronic phase lasts within 3–5 years. After eventual transformation into accelerated and/or blastic phases, it generally become to be fatal.[52] CML cells have Philadelphia chromosome abnormality, a unique t(9;22)(q34;q11), that invents a BCR-ABL fusion oncoprotein.[52] Constitutively active BCR-ABL promotes CML-specific phenotypes, such as leukocytosis with immature to mature cells, thrombocytosis, and splenomegaly, through influencing the Ras/MAPK, Janus kinase (JAK)-STAT, PI3K/Akt, and NF-κB pathways.[52] Since tyrosine kinase inhibitors (TKIs) to block BCR-ABL activity was approved for the treatment of CML, its clinical outcome has remarkably improved. Nevertheless, approximately half of patients experience molecular relapse within one year after stopping TKI therapy, even if they have achieved deep molecular remission.[53] CML patients with lifelong TKI-treatment are confronted with difficulties in decline of QOL from adverse reactions as well as escalation of the costs of treatment.

Recent clinical experiments have suggested that inhibition of BCR-ABL kinase activity alone fails to completely eliminate CML LSCs.[54] Indeed, a distinct subset of CML LSCs with a deep quiescent signature is persistently detected even when TKI-induced remission.[55] Survival of these primitive CML LSCs is not dependent on BCR-ABL1 activity alone.[56] Transforming growth factor-β (TGF-β), Foxo, Hedgehog, Wnt, and JAK/STAT signaling are proposed to be involved in the survival and self-renewal of CML LSCs during TKI-treatment; in addition, bone marrow microenvironment also functions to sustain CML LSCs. Thus, understanding of molecular mechanisms to maintain LSCs is required to develop novel therapeutic strategies for CML.

STAP-2 interacts with BCR-ABL dependently of its SH2 domain.[17] BCR-ABL phosphorylates STAP-2 Tyr250, and phosphorylated STAP-2 in turn upregulates BCR-ABL phosphorylation, resulted in elevated activation of ERK and STAT5 as well as enhanced gene expression of BCL-2 and BCL-xL (Fig. 4E). Interactions between STAP-2 and BCR-ABL also induce changes of expression pattern of chemokine receptors, with CXCR4 downregulation and CCR7 upregulation.[17] Binding of STAP-2 to BCR-ABL is involved in conferring a growth advantage and resistance to TKIs, as well as disease progression.[17] Of note, mice injected with BCR-ABL/STAP-2-expressing Ba/F3 pro-B cells show severe hepatosplenomegaly and lymph node swelling.[17] In addition, knockdown of STAP-2 expression in K562 CML cells abolish tumor formation capacity in injected mice.[17] These findings indicate crucial involvement of STAP-2 in BCR-ABL activity, and propose that STAP-2 may be a powerful candidate to develop new drugs for CML patients. The expression profile of STAP-2 may provide important information to estimate characteristics of individual CML clones.

Recently, STAP-1 has been reported to be involved in CML pathogenesis.[14] STAP-1 gene expression is aberrantly high in CML LSCs from patient's bone marrow. Mice injected with STAP-1-deficient CML LSCs live longer through increased apoptosis in CML LSCs than those with wild-type CML LSCs. When STAP-1 gene is ablated, insufficient STAT5 activation leads to downregulation of gene expression of anti-apoptotic BCL-2 and BCL-xL. Recent transcriptome analyses have reported that STAP-1 influences some signaling pathways related to BCR-ABL, peroxisome proliferator-activated receptor γ (PPARγ), and JAK2. Regarding mechanisms for the effect of STAP-1 on CML cells, STAP-1 binds to both BCR-ABL and STAT5a through its SH2 and PH domains, respectively; therefore, STAP-1 is likely to act as a scaffold protein[17,57] (Fig. 4F). In CML cells, the binding between STAP-1 and BCR-ABL also stabilizes BCR-ABL protein.[57] Importantly, a transcriptional factor, nuclear factor of activated T-cells (NFAT)c1 binds to and induces activation of the STAP-1 promoter[57] (Fig. 4F). Therefore, STAP-1 positively regulates BCR-ABL/STAT5 and Ca^{2+}/NFAT signals, leading to high capacity of cell growth as well as STAP-1 mRNA expression in CML cells. Because STAP-1 has little influence on normal hematopoiesis under steady condition,[51] STAP-1 inhibition seems to be a suitable therapeutic strategy for the treatment of CML through inhibiting the BCR-BAL/STAT5 axis. Alternatively, targeting the Ca^{2+}/NFAT pathway may have potential to inhibit STAP-1 expression in the BCR-ABL/STAP-1 loop in CML cells. Hopefully, STAP inhibitors will be developed and used to treat CML patients with overcoming resistance and disease persistence.

Besides CML, a report described possible involvement of STAP-1 in hematological malignancies. STAP-1 mRNAs are highly expressed in pediatric B-cell precursor acute lymphoblastic leukemia.[58] Microarray data from 572 patients indicate that STAP-1 expression is elevated in approximately 20% of samples independently of the presence of the BCR-ABL fusion gene. Therefore, STAP-1 may be a possible target in another BCR-ABL-related disease, such as acute lymphoblastic leukemia.

3. CONCLUSION

We have summarized functions of STAP adaptor proteins in cancers. STAP proteins have roles in several types of cancers. STAP-2 is involved in cell growth of breast and prostate cancers through enhancing STAT3 activation. STAP-2 regulates the tyrosinase protein level in melanoma, which determines tumor cell infiltration into organs within the body. STAP-2 modulates EBV LMP1-mediated NF-κB activation in malignant lymphomas. Both STAP-1 and STAP-2 are involved in CML pathogenesis by interacting with BCR-ABL protein.

The genomic sequence within 2 kb of the 5′ flanking region of the STAP-2 transcription initiation site contains putative binding sites for c-Rel, AP-1, p65/NF-κB, and STAT proteins,[7] that are often activated by bacterial pathogens and inflammatory cytokines, including IL-1, TNF-α, and IL-6. Indeed, STAP-2 expression is induced by L-6- and TNF-α-stimulation. Viral LMP1 protein and bacterial pathogens, such as lipopolysaccharide (LPS), also induce STAP-2 expression through NF-κB activation. In turn, STAP-2 enhances the activity of STAT3 and NF-κB. Both NF-κB and STAT3 are central hubs in signals for oncogenesis and inflammation. NF-κB regulates gene expression for anti-apoptosis as well as pro-inflammatory cytokines and chemokines, and constitutively active NF-κB is reported in various types of cancers.[59] Targeting of NF-κB or STAT3 gene is actually related to tumor cell growth, migration, and invasion. Thus, STAP-2 is likely to associate with the STAT3/NF-κB axis during tumorigenesis. Therefore, it is very informative to clarify regulatory mechanisms for STAPs in malignant cells. Furthermore, detailed molecular interactions between STAPs and signaling and/or transcriptional molecules will provide clues toward development of novel drugs for cancer therapy in the near future.

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Conflict of Interest The authors declare no conflict of interest.

REFERENCES

1) Sever R, Brugge JS. Signal transduction in cancer. *Cold Spring Harb. Perspect. Med*, **5**, a006098 (2015).
2) Adjei AA, Hidalgo M. Intracellular signal transduction pathway proteins as targets for cancer therapy. *J. Clin. Oncol.*, **23**, 5386–5403 (2005).
3) Di Domenico M, Giordano A. Signal transduction growth factors: the effective governance of transcription and cellular adhesion in cancer invasion. *Oncotarget*, **8**, 36869–36884 (2017).
4) Naudin C, Chevalier C, Roche S. The role of small adaptor proteins in the control of oncogenic signaling driven by tyrosine kinases in human cancer. *Oncotarget*, **7**, 11033–11055 (2016).
5) Ohya K, Kajigaya S, Kitanaka A, Yoshida K, Miyazato A, Yamashita Y, Yamanaka T, Ikeda U, Shimada K, Ozawa K, Mano H. Molecular cloning of a docking protein, BRDG1, that acts downstream of the Tec tyrosine kinase. *Proc. Natl. Acad. Sci. U.S.A.*, **96**, 11976–11981 (1999).
6) Masuhara M, Nagao K, Nishikawa M, Sasaki M, Yoshimura A, Osawa M. Molecular cloning of murine STAP-1, the stem-cell-specific adaptor protein containing PH and SH2 domains. *Biochem. Biophys. Res. Commun.*, **268**, 697–703 (2000) [PMID: 10679268 DOI: 10.1006/bbrc.2000.2223].
7) Matsuda T, Oritani K. STAP-2 adaptor protein regulates multiple steps of immune and inflammatory responses. *Biol. Pharm. Bull.*, **44**, 895–901 (2021).
8) Stoecker K, Weigelt K, Ebert S, Karlstetter M, Walczak Y, Langmann T. Induction of STAP-1 promotes neurotoxic activation of microglia. *Biochem. Biophys. Res. Commun.*, **379**, 121–126 (2009).
9) Fouchier SW, Dallinga-Thie GM, Meijers JC, Zelcer N, Kastelein JJ, Defesche JC, Hovingh GK. Mutations in STAP1 are associated with autosomal dominant hypercholesterolemia. *Circ. Res.*, **115**, 552–555 (2014).
10) Blanco-Vaca F, Martin-Campos JM, Perez A, Fuentes-Prior P. A rare STAP1 mutation incompletely associated with familial hypercholesterolemia. *Clin. Chim. Acta*, **487**, 270–274 (2018).
11) Lamiquiz-Moneo I, Restrepo-Cordoba MA, Mateo-Gallego R, Bea AM, Del Pino Alberiche-Ruano M, Garcia-Pavia P, Cenarro A, Martin C, Civeira F, Sánchez-Hernández RM. Predicted pathogenic mutations in STAP1 are not associated with clinically defined familial hypercholesterolemia. *Atherosclerosis*, **292**, 143–151 (2020).
12) Loaiza N, Hartgers ML, Reeskamp LF, Balder JW, Rimbert A, Bazioti V, Wolters JC, Winkelmeijer M, Jansen HPG, Dallinga-Thie GM, Volta A, Huijkman N, Smit M, Kloosterhuis N, Koster M, Svendsen AF, van de Sluis B, Hovingh GK, Grefhorst A, Kuivenhoven JA. Taking one step back in familial hypercholesterolemia: STAP1 does not alter plasma LDL (Low-Density Lipoprotein) cholesterol in mice and humans. *Arterioscler. Thromb. Vasc. Biol.*, **40**, 973–985 (2020).
13) Kashiwakura JI, Saitoh K, Ihara T, Sasaki Y, Kagohashi K, Enohara S, Morioka Y, Watarai H, Muromoto R, Kitai Y, Iwabuchi K, Oritani K, Matsuda T. Expression of signal-transducing adaptor protein-1 attenuates experimental autoimmune hepatitis *via* down-regulating activation and homeostasis of invariant natural killer T cells. *PLOS ONE*, **15**, e0241440 (2020).
14) Toda J, Ichii M, Oritani K, Shibayama H, Tanimura A, Saito H, Yokota T, Motooka D, Okuzaki D, Kitai Y, Muromoto R, Kashiwakura JI, Matsuda T, Hosen N, Kanakura Y. Signal-transducing adapter protein-1 is required for maintenance of leukemic stem cells in CML. *Oncogene*, **39**, 5601–5615 (2020).
15) Ikeda O, Sekine Y, Mizushima A, Nakasuji M, Miyasaka Y, Yamamoto C, Muromoto R, Nanbo A, Oritani K, Yoshimura A, Matsuda T. Interactions of STAP-2 with Brk and STAT3 participate in cell growth of human breast cancer cells. *J. Biol. Chem.*, **285**, 38093–38103 (2010).
16) Sekine Y, Togi S, Muromoto R, Kon S, Kitai Y, Yoshimura A, Oritani K, Matsuda T. STAP-2 protein expression in B16F10 melanoma cells positively regulates protein levels of tyrosinase, which determines organs to infiltrate in the body. *J. Biol. Chem.*, **290**, 17462–17473 (2015).
17) Sekine Y, Ikeda O, Mizushima A, Ueno Y, Muromoto R, Yoshimura A, Kanakura Y, Oritani K, Matsuda T. STAP-2 interacts with and modulates BCR-ABL-mediated tumorigenesis. *Oncogene*, **31**, 4384–4396 (2012).
18) Harbeck N, Penault-Llorca F, Cortes J, Gnant M, Houssami N,

Poortmans P, Ruddy K, Tsang J, Cardoso F. Breast cancer. *Nat. Rev. Dis. Primers*, **5**, 66 (2019).
19) Marra A, Trapani D, Viale G, Criscitiello C, Curigliano G. Practical classification of triple-negative breast cancer: intratumoral heterogeneity, mechanisms of drug resistance, and novel therapies. *NPJ Breast Cancer*, **6**, 54 (2020).
20) Brauer PM, Tyner AL. Building a better understanding of the intracellular tyrosine kinase PTK6—BRK by BRK. *Biochim. Biophys. Acta*, **1806**, 66–73 (2010).
21) Miah S, Bagu E, Goel R, Ogunbolude Y, Dai C, Ward A, Vizeacoumar FS, Davies G, Vizeacoumar FJ, Anderson D, Lukong KE. Estrogen receptor signaling regulates the expression of the breast tumor kinase in breast cancer cell. *BMC Cancer*, **19**, 78 (2019).
22) Born M, Quintanilla-Fend L, Braselmann H, Reich U, Richter M, Hutzler P, Aubele M. Simultaneous over-expression of the Her2/neu and PTK6 tyrosine kinases in archival invasive ductal breast carcinomas. *J. Pathol.*, **205**, 592–596 (2005).
23) Grimley PM, Dong F, Rui H. Stat5a and Stat5b: fraternal twins of signal transduction and transcriptional activation. *Cytokine Growth Factor Rev.*, **10**, 131–157 (1999).
24) Bowman T, Garcia R, Turkson J, Jove R. STATs in oncogenesis. *Oncogene*, **19**, 2474–2488 (2000).
25) Weaver AM, Silva CM. Signal transducer and activator of transcription 5b: a new target of breast tumor kinase/protein tyrosine kinase 6. *Breast Cancer Res.*, **9**, R79 (2007).
26) Rebello RJ, Oing C, Knudsen KE, Loeb S, Johnson DC, Reiter RE, Gillessen S, Van der Kwast T, Bristow RG. Prostate cancer. *Nat. Rev. Dis. Primers*, **7**, 9 (2021).
27) Gerber DE. EGFR inhibition in the treatment of non-small cell lung cancer. *Drug Dev. Res.*, **69**, 359–372 (2008).
28) Canil CM, Moore MJ, Winquist E, Baetz T, Pollak M, Chi KN, Berry S, Ernst DS, Douglas L, Brundage M, Fisher B, McKenna A, Seymour L. Randomized phase II study of two doses of gefitinib in hormone-refractory prostate cancer: a trial of the National Cancer Institute of Canada-Clinical Trials Group. *J. Clin. Oncol.*, **23**, 455–460 (2005).
29) Kitai Y, Iwakami M, Saitoh K, Togi S, Isayama S, Sekine Y, Muromoto R, Kashiwakura JI, Yoshimura A, Oritani K, Matsuda T. STAP-2 protein promotes prostate cancer growth by enhancing epidermal growth factor receptor stabilization. *J. Biol. Chem.*, **292**, 19392–19399 (2017).
30) Heo T-H, Wahler J, Suh N. Potential therapeutic implications of IL-6/IL-6R/gp130-targeting agents in breast cancer. *Oncotarget*, **7**, 15460–15473 (2016).
31) Li X, Lu Y, Liang K, Hsu JM, Albarracin C, Mills GB, Hung MC, Fan Z. Brk/PTK6 sustains activated EGFR signaling through inhibiting EGFR degradation and transactivating EGFR. *Oncogene*, **31**, 4372–4383 (2012).
32) Carrión-Salip D, Panosa C, Menendez JA, Puig T, Oliveras G, Pandiella A, De Llorens R, Massaguer A. Androgen-independent prostate cancer cells circumvent EGFR inhibition by overexpression of alternative HER receptors and ligands. *Int. J. Oncol.*, **41**, 1128–1138 (2012).
33) Ossio R, Roldan-Marín R, Martinez-Said H, Adams DJ, Robles-Espinoza CD. Melanoma: a global perspective. *Nat. Rev. Cancer*, **17**, 393–394 (2017).
34) Schadendorf D, Fisher DE, Garbe C, Gershenwald JE, Grob JJ, Halpern A, Herlyn M, Marchetti MA, McArthur G, Ribas A, Roesch A, Hauschild A. Melanoma. *Nat. Rev. Dis. Primers*, **1**, 15003 (2015).
35) Flaherty KT, Robert C, Hersey P, *et al.* Improved survival with MEK inhibition in BRAF-mutated melanoma. *N. Engl. J. Med.*, **367**, 107–114 (2012).
36) Fidler IJ. Biological behavior of malignant melanoma cells correlated to their survival *in vivo*. *Cancer Res.*, **35**, 218–224 (1975).
37) Halaban R, Moellmann G, Tamura A, Kwon BS, Kuklinska E, Pomerantz SH, Lerner AB. Tyrosinases of murine melanocytes with mutations at the albino locus. *Proc. Natl. Acad. Sci. U.S.A.*, **85**, 7241–7245 (1988).
38) Tomita Y. The molecular genetics of albinism and piebaldism. *Arch. Dermatol.*, **130**, 355–358 (1994).
39) Kang JS, Cho D, Kim YI, Hahm E, Yang Y, Kim D, Hur D, Park H, Bang S, Hwang YI, Lee WJ. L-ascorbic acid (vitamin C) induces the apoptosis of B16 murine melanoma cells *via* a caspase-8-independent pathway. *Cancer Immunol. Immunother.*, **52**, 693–698 (2003).
40) Kühnelt-Leddihn L, Müller H, Eisendle K, Zelger B, Weinlich G. Overexpression of the chemokine receptors CXCR4, CCR7, CCR9, and CCR10 in human primary cutaneous melanoma: a potential prognostic value for CCR7 and CCR10. *Arch. Dermatol. Res.*, **304**, 185–193 (2012).
41) Wiley HE, Gonzalez EB, Maki W, Wu MT, Hwang ST. Expression of CC chemokine receptor-7 and regional lymph node metastasis of B16 murine melanoma. *J. Natl. Cancer Inst.*, **93**, 1638–1643 (2001).
42) Murakami T, Maki W, Cardones AR, Fang H, Tun Kyi A, Nestle FO, Hwang ST. Expression of CXC chemokine receptor-4 enhances the pulmonary metastatic potential of murine B16 melanoma cells. *Cancer Res.*, **62**, 7328–7334 (2002).
43) Kawada K, Sonoshita M, Sakashita H, Takabayashi A, Yamaoka Y, Manabe T, Inaba K, Minato N, Oshima M, Taketo MM. Pivotal role of CXCR3 in melanoma cell metastasis to lymph nodes. *Cancer Res.*, **64**, 4010–4017 (2004).
44) Thorley-Lawson DA. Epstein–Barr virus: exploiting the immune system. *Nat. Rev. Immunol.*, **1**, 75–82 (2001).
45) Ikeda O, Sekine Y, Yasui T, Oritani K, Sugiyma K, Muromoto R, Ohbayashi N, Yoshimura A, Matsuda T. STAP-2 negatively regulates both canonical and noncanonical NF-kappaB activation induced by Epstein–Barr virus-derived latent membrane protein 1. *Mol. Cell. Biol.*, **28**, 5027–5042 (2008).
46) Li Q, Verma IM. NF-kappaB regulation in the immune system. *Nat. Rev. Immunol.*, **2**, 725–734 (2002).
47) Jost PJ, Ruland J. Aberrant NF-kappaB signaling in lymphoma: mechanisms, consequences, and therapeutic implications. *Blood*, **109**, 2700–2707 (2007).
48) Chaudhary PM, Jasmin A, Eby MT, Hood L. Modulation of the NF-kappa B pathway by virally encoded death effector domains-containing proteins. *Oncogene*, **18**, 5738–5746 (1999).
49) Tanaka A, Takahashi C, Yamaoka S, Nosaka T, Maki M, Hatanaka M. Oncogenic transformation by the tax gene of human T-cell leukemia virus type I *in vitro*. *Proc. Natl. Acad. Sci. U.S.A.*, **87**, 1071–1075 (1990).
50) Grossman WJ, Kimata JT, Wong FH, Zutter M, Ley TJ, Ratner L. Development of leukemia in mice transgenic for the tax gene of human T-cell leukemia virus type I. *Proc. Natl. Acad. Sci. U.S.A.*, **92**, 1057–1061 (1995).
51) Ichii M, Oritani K, Toda J, Saito H, Shi H, Shibayama H, Motooka D, Kitai Y, Muromoto R, Kashiwakura JI, Saitoh K, Okuzaki D, Matsuda T, Kanakura Y. Signal-transducing adaptor protein-2 delays recovery of B lineage lymphocytes during hematopoietic stress. *Haematologica*, **106**, 424–436 (2021).
52) Quintás-Cardama A, Cortes J. Molecular biology of bcr-abl1-positive chronic myeloid leukemia. *Blood*, **113**, 1619–1630 (2009).
53) Mahon FX, Réa D, Guilhot J, Guilhot F, Huguet F, Nicolini F, Legros L, Charbonnier A, Guerci A, Varet B, Etienne G, Reiffers J, Rousselot P. Discontinuation of imatinib in patients with chronic myeloid leukaemia who have maintained complete molecular remission for at least 2 years: the prospective, multicentre Stop Imatinib (STIM) trial. *Lancet Oncol.*, **11**, 1029–1035 (2010).
54) Lemoli RM, Salvestrini V, Bianchi E, Bertolini F, Fogli M, Amabile M, Tafuri A, Salati S, Zini R, Testoni N, Rabascio C, Rossi L, Martin-Padura I, Castagnetti F, Marighetti P, Martinelli G, Baccarani M, Ferrari S, Manfredini R. Molecular and functional analysis of

the stem cell compartment of chronic myelogenous leukemia reveals the presence of a $CD34^-$ cell population with intrinsic resistance to imatinib. *Blood*, **114**, 5191–5200 (2009).

55) Giustacchini A, Thongjuea S, Barkas N, Woll PS, Povinelli BJ, Booth CAG, Sopp P, Norfo R, Rodriguez-Meira A, Ashley N, Jamieson L, Vyas P, Anderson K, Segerstolpe A, Qian H, Olsson-Strömberg U, Mustjoki S, Sandberg R, Jacobsen SEW, Mead AJ. Single-cell transcriptomics uncovers distinct molecular signatures of stem cells in chronic myeloid leukemia. *Nat. Med.*, **23**, 692–702 (2017).

56) Hamilton A, Helgason GV, Schemionek M, Zhang B, Myssina S, Allan EK, Nicolini FE, Müller-Tidow C, Bhatia R, Brunton VG, Koschmieder S, Holyoake TL. Chronic myeloid leukemia stem cells are not dependent on Bcr-Abl kinase activity for their survival. *Blood*, **119**, 1501–1510 (2012).

57) Ishiura M, Kitai Y, Kashiwakura JI, Muromoto R, Toda J, Ichii M, Oritani K, Matsuda T. Positive interactions between STAP-1 and BCR-ABL influence chronic myeloid leukemia cell proliferation and survival. *Biochem. Biophys. Res. Commun.*, **556**, 185–191 (2021).

58) Steeghs EMP, Bakker M, Hoogkamer AQ, Boer JM, Hartman QJ, Stalpers F, Escherich G, de Haas V, de Groot-Kruseman HA, Pieters R, den Boer ML. High STAP1 expression in DUX4-rearranged cases is not suitable as therapeutic target in pediatric B-cell precursor acute lymphoblastic leukemia. *Sci. Rep.*, **8**, 693 (2018).

59) Darnell JE Jr. Transcription factors as targets for cancer therapy. *Nat. Rev. Cancer*, **2**, 740–749 (2002).