CRMP5 Controls Glioblastoma Cell Proliferation and Survival through Notch-Dependent Signaling

Aubin Moutal1, Jérôme Honnorat1, Patrick Massoma1, Pauline Désormeaux1, Caroline Bertrand1, Céline Malleval1, Chantal Watrin1, Naura Chounlamountri1, Marie-Eve Mayeur1, Roger Besançon1, Nicolas Naudet1, Léa Magadoux1, Rajesh Khanna2, François Ducray1,2, David Meyronet1,4, and Nicole Thomasset1

Abstract

Collapsin response mediator protein 5 (CRMP5) belongs to a family of five cytosolic proteins that play a major role in nervous system development. This protein was first described in cancer-induced autoimmune processes, causing neurodegenerative disorders (paraneoplastic neurologic syndromes). CRMP5 expression has been reported to serve as a biomarker for high-grade lung neuroendocrine carcinomas; however, its functional roles have not been examined in any setting of cancer pathophysiology. In this study, we report two different CRMP5 expression patterns observed in human glioblastoma (GBM) biopsies that establish connections between CRMP5 expression, Notch receptor signaling, and GBM cell proliferation. We demonstrated that elevated CRMP5 promotes Notch receptor expression and Akt activation in human tumor cell lines, GBM stem cells, and primary tumor biopsies. We have shown that the high CRMP5 and Notch expression in GBM xenograft is related to stem cells. This suggests that high CRMP5 expression pattern in GBM biopsies encompasses a subset of stem cells. Mechanistically, CRMP5 functioned by hijacking Notch receptors from Itch-dependent lysosomal degradation. Our findings suggest that CRMP5 serves as a major mediator of Notch signaling and Akt activation by controlling the degradation of the Notch receptor, with implications for defining a biomarker signature in GBM that correlates with and may predict patient survival.

Introduction

Glioblastoma (GBM) is the most frequent and malignant primary tumor of the central nervous system (1). Median survival time after diagnosis is approximately 10.5 months (2) despite breakthroughs in clinical research uncovering a number of genetic and protein abnormalities; among them, deregulation of signal transduction pathways and loss of cell-cycle control are prominent (3, 4).

Collapsin response mediator proteins (CRMP; CRMP-1 to -5) are multifunctional proteins, highly expressed in the developing brain and contribute to neuronal polarity establishment (5). Altered CRMP levels were detected in several cancers, including breast, colorectal, prostate, pancreatic, and neuroendocrine lung carcinoma (6). CRMPs are found broadly expressed within tissues and the understanding of their cellular functions is expanding. In dividing cells, such as lung cancer cells, CRMP2, the best-studied isoform of the family, has been involved in cell division alteration and growth control (7). The most recently identified CRMP, CRMP5 (8–10), is widely expressed in adult brain regions of neurogenesis (11). We previously described that CRMP5 protein expression in peripheral tumors causes the development of paraneoplastic neurologic syndromes (10, 12, 13). Furthermore, we proposed CRMP5 protein expression as a marker for high-grade lung neuroendocrine carcinomas, a very aggressive and treatment-resistant lung tumor (14) and observed its expression in GBM cells (15). However, no specific role has been proposed for CRMP5 protein in GBM, or, more broadly, in the cancer process. Interestingly, in Drosophila, a CRMP homologue was recently described as promoting Notch signaling during asymmetric division of sensory progenitors (16), suggesting the possibility that CRMP5 and the Notch signaling pathway may converge in GBM proliferation.

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

D. Meyronet and N. Thomasset contributed equally to this article.

Corresponding Author: Nicole Thomasset, INSERM U1028, CNRS UMR5292, Lyon Neuroscience Research Center, Neuro-oncology and Neuro-Inflammation Team, University Claude Bernard Lyon 1, Lyon, France.

Collapsin response mediator proteins (CRMP; CRMP-1 to -5) are multifunctional proteins, highly expressed in the developing brain and contribute to neuronal polarity establishment (5). Altered CRMP levels were detected in several cancers, including breast, colorectal, prostate, pancreatic, and neuroendocrine lung carcinoma (6). CRMPs are found broadly expressed within tissues and the understanding of their cellular functions is expanding. In dividing cells, such as lung cancer cells, CRMP2, the best-studied isoform of the family, has been involved in cell division alteration and growth control (7). The most recently identified CRMP, CRMP5 (8–10), is widely expressed in adult brain regions of neurogenesis (11). We previously described that CRMP5 protein expression in peripheral tumors causes the development of paraneoplastic neurologic syndromes (10, 12, 13). Furthermore, we proposed CRMP5 protein expression as a marker for high-grade lung neuroendocrine carcinomas, a very aggressive and treatment-resistant lung tumor (14) and observed its expression in GBM cells (15). However, no specific role has been proposed for CRMP5 protein in GBM, or, more broadly, in the cancer process. Interestingly, in Drosophila, a CRMP homologue was recently described as promoting Notch signaling during asymmetric division of sensory progenitors (16), suggesting the possibility that CRMP5 and the Notch signaling pathway may converge in GBM proliferation.

Notch signaling pathway activation has been described in GBM as a major pathway mediating proliferation and survival (17, 18), cell fate decisions (19), angiogenesis (20), and resistance to therapy (21, 22). Moreover, ectopic Notch activation affects other signaling pathways, such as Akt, which play a major oncogenic role in GBM growth (23, 24). Reinforcing the role of the Notch pathway in this tumor type, Notch1 protein expression is correlated with tumor progression and is an independent predictor of poor survival in glioma patients (25, 26). Notch receptor activation is context-dependent and requires several cleavages, which subsequently led to translocation of its cytoplasmic domain to the nucleus to promote Notch-targeted genes, such as the Hes/Hey
basic helix–loop–helix (bHLH) transcription factors and cyclin D1, a G1/S cell-cycle phase transition marker (27, 28). However, how Notch signaling pathway is regulated at various functional levels is unknown.

Here, we demonstrate that CRMP5 is an indicator of poor survival in 183 patients and promotes GBM cell proliferation in a Notch/Akt–dependent manner. We report an unexpected role for CRMP5 in facilitating Notch signaling and Akt activation, leading to sustained GBM proliferation by preventing Notch lysosomal degradation. This study reveals a new pathophysiologic mechanism regulating GBM proliferation. We anticipate potential utility of CRMP5 protein as a biomarker in the development of pretreatment screening or follow-up programs.

Materials and Methods

GBM patient cohort

GBM specimens were obtained from Hospices Civils de Lyon, Centre de Pathologie et Neuropathologie Est, Cardiobiotec and Neurobiotec (Lyon, France) in compliance with French ethical rules. After pathologist reviews, we studied 183 patients diagnosed as GBM according to World Health Organization (1, 2, 29). No IDH1 R132H mutation was retrieved in this series using nondenosed as GBM according to World Health Organization (1, 2, 29).

Primary cultures, cell lines, transfection, and reagents

Human GBMs primary cultures were obtained from biopsies (Hospices Civils de Lyon) or xenografts in nude mice from human GBM patient cohort

Materials and Methods

GBM specimens were obtained from Hospices Civils de Lyon, Centre de Pathologie et Neuropathologie Est, Cardiobiotec and Neurobiotec (Lyon, France) in compliance with French ethical rules. After pathologist reviews, we studied 183 patients diagnosed as GBM according to World Health Organization (1, 2, 29). No IDH1 R132H mutation was retrieved in this series using nondenosed as GBM according to World Health Organization (1, 2, 29).

Primary cultures, cell lines, transfection, and reagents

Human GBMs primary cultures were obtained from biopsies (Hospices Civils de Lyon) or xenografts in nude mice from human GBM patient cohort

Results

CRMP5 protein is expressed in human GBM and established GBM-derived cell lines

We examined CRMP5 protein expression via Western blot analyses in human GBM tumor samples, primary cultures, cell lines, and xenografts. CRMP5 was detected in human GBM tumor samples (GBM 7, 8, 9, and 10), primary GBM cultures (cult 2, 3, 4, 6, and 8), both GL15 and A172 GBM cell lines (Fig. 1A) and in GBM_SC1 xenografts but not in GBM_SC2 xenograft (Fig. 1B). Subcellular fractionation of GBM extracts was performed to determine the subcellular localization of CRMP5 in human GBM tumors.
CRMP5 Promotes Notch-Dependent Proliferation in GBM

Figure 1. CRMP5 is expressed in the cytoplasm and nucleus of GBM cells. A, CRMP5 expression was detected by Western blot analysis of GBM tumors (GBM-7 to -10), primary cultures (Cult 2 to Cult 8), and GL15 and A172 cell lines, with anti-GAPDH as a loading control. B, CRMP5 expression in three different passages in GBM_SC1 and GBM_SC2 cell cultured from GBM subcutaneous xenografts in nude mice using Western blot analysis, with actin expression as a loading control. C, immunofluorescence against CRMP5: cytoplasmic and nuclear localization in GL15 and A172 cell lines and in human GBM biopsy section; DAPI stains the nucleus. Scale bar, 15 μm. Immunoperoxidase technique against CRMP5 in xenograft models GBM_SC1 and GBM_SC2 using. Scale bar, 10 μm.

CRMP5 controls GBM cell proliferation in vitro and in vivo

The potential role of CRMP5 in GBM proliferation was further evaluated in GBM primary cultures and cell lines after CRMP5 knockdown or overexpression using the 5-ethyl-2'-deoxuryridine (EdU) incorporation assay. Two completely different CRMP5 siRNA sequences (siRNA1 and siRNA2) knocked down CRMP5 expression in a GBM primary culture SC1 and in GL15 and A172 cells. CRMP5 silencing by CRMP5-siRNA1 led to a significantly lower mean proliferation index, which was decreased by 74.3%, 59.4%, and 89.7% in the GBM primary culture SC1 and in GL15 and A172 cells, respectively, compared with control (Fig. 3A and B). Similar results were obtained CRMP5-siRNA2 (Supplementary Fig. S3A and S3B), validating the specificity and lack of off-target effect of the knockdown. As a corollary, CRMP5 overexpression in GL15 and A172 cells increased their proliferation index by 55% and 45%, respectively, compared with control cells transfected with empty plasmid (Fig. 3B). Because a defect of proliferation could be caused by an alteration of cell-cycle progression, we measured the DNA content of GL15 cells after CRMP5 knockdown (Supplementary Fig. S4A and S4B). CRMP5 depletion led to a significantly lower mean mitotic index, which was decreased by 55% and 45%, respectively, compared with control cells (Fig. 3B). These results were reproduced using CRMP5-siRNA2 (Supplementary Fig. S3B and S3C). Finally, we did not observe any significant effect on apoptosis using a TUNEL assay on GL15 cells after CRMP5 depletion (Supplementary Fig. S4A–S4C).

CRMP5 is expressed in the cytoplasm and nucleus of GBM cells. A, CRMP5 expression was detected by Western blot analysis of GBM tumors (GBM-7 to -10), primary cultures (Cult 2 to Cult 8), and GL15 and A172 cell lines, with anti-GAPDH as a loading control. B, CRMP5 expression in three different passages in GBM_SC1 and GBM_SC2 cell cultured from GBM subcutaneous xenografts in nude mice using Western blot analysis, with actin expression as a loading control. C, immunofluorescence against CRMP5: cytoplasmic and nuclear localization in GL15 and A172 cell lines and in human GBM biopsy section; DAPI stains the nucleus. Scale bar, 15 μm. Immunoperoxidase technique against CRMP5 in xenograft models GBM_SC1 and GBM_SC2 using. Scale bar, 10 μm.
that tumor sizes had to be compared at 21 days after injection. Mean tumor volumes were found to be significantly higher for GBM_SC1 (1,585 mm³, n = 24) than for GBM_SC2 (781 mm³, n = 20, P < 0.0001, Student t test), showing that CRMP5 expression is related to GBM growth in vivo (Fig. 3E and Supplementary Fig. S3D).

CRMP5 is involved in Akt activation and Notch expression

GBM cell proliferation has been extensively correlated to Notch signaling pathway activation (32–34). Because Notch controls cyclin D1 expression and Akt activation to promote GBM proliferation (34), we examined a possible connection between CRMP5 expression, Notch receptors expression and Akt activation. First, using calcium depletion by EDTA as previously documented (35), we observed efficient, endogenous activation of Notch signaling in GL15 cells, as evidenced by increased nuclear Notch localization (Supplementary Fig. S5A). After treatment with DAPT, a γ-secretase inhibitor, Notch accumulated at the cell membrane (Supplementary Fig. S5D). CRMP5 knockdown resulted in a loss of Notch1 and Notch2 expression in the GL15 cell line (Fig. 4A). We next tested whether Akt activation could be impaired by concomitant loss of CRMP5 and Notch receptors. Although Akt expression was not affected, its activation, monitored by detecting the S473-phosphorylation site, was lost. Identical results were obtained using A172 cells (Fig. 4A). In contrast, overexpression of a GFP-tagged CRMP5 in GL15 and A172 cells induced robust Notch1 and Notch2 expression that was concomitant with an increase of Akt phosphorylation (Fig. 4B). Our findings suggest that CRMP5 regulates the expression of Notch1 and Notch2 receptors and controls Akt activation as a Notch downstream target. Corollary to these results, we showed in GBM_SC1 xenograft cells grown in neurospheres that the CRMP5⁺ cells coexpressed both Nestin⁺ (by 94.7%) and IQGAP1⁺ (by 98.6%), as well as Notch1⁺ that are GBM stem cells markers (Supplementary Fig. S8A). IQGAP1 specified a subpopulation of amplifying tumor cells with proliferative features in GBM-like tumors (36). Although the GBM_SC1 neurospheres expressed neural stem cell markers such as CD133, Musashi1, IQGAP1, Nestin, Notch1, Notch2, as well as CRMP5, we observed the extinction of these proteins expressions when neurospheres shifted to differentiated non-stem cells in differentiation medium (Supplementary Fig. S8B and S8C). The concomitant loss of CRMP5, Hes, and p-Akt expressions in differentiated non-stem cells grown in differentiation medium showed that Notch signaling activation and Akt phosphorylation were positively correlated to CRMP5 expression in GBM_SC1 stem cells (Supplementary Fig. S8C).

To further investigate the relationship between CRMP5 and Notch in tumors, we assessed Notch expression in lysates from CRMP5LOW or CRMP5HIGH GBM frozen biopsies. Consistent with these in vitro data, Notch1 and Notch2 were mostly detected in the CRMP5HIGH GBMs, in which Akt phosphorylation was found to be strong compared with CRMP5LOW GBMs. However, Akt total expression was similar in both GBM subtypes (Fig. 4C) immunohistochemical characterization of Notch1 expression in GBM biopsies revealed two different Notch1 expression patterns that paralleled the two CRMP5 expression patterns (Fig. 4D). The percentage of GBM tumors expressing Notch1 was higher in the CRMP5HIGH group than in the CRMP5LOW group [94% (15 of 16) vs. 46% (10 of 22); P = 0.0018, χ² test; Fig. 4E, top]. Interestingly, all GBMs with nuclear Notch1 staining also displayed cytoplasmic staining; however, the opposite scenario did not hold true. Therefore, we assessed the fraction of GBM tumors with Notch1 nuclear staining showing activation of the receptor (37). The percentage of GBM tumors with Notch1 nuclear staining was also higher in the CRMP5HIGH group than in the CRMP5LOW group [88% (14 of 16) vs. 23% (5 of 22); P < 0.0001, χ² test; graph not shown]. Furthermore, the percentage of GBM tumors with Notch1-positive mitotic figures was also significantly higher in the...
CRMP5 expression in human GBM is related to Notch pathway and Akt activation.

CRMP5 positively modulates Notch signaling activation in GBM

Upon activation by its ligand, Notch receptors are ultimately translocated into the nucleus to promote the transcriptional activity of target genes such as the hairy/enhancer of split (hes) genes (38). To study whether CRMP5 could control hes1 gene transcription, we transiently transfected GL15 cells with a luciferase reporter construct driven by the hes1 promoter together with CRMP5-siRNA or control-siRNA. CRMP5-siRNA inhibited luciferase activity (normalized to Renilla luciferase activity) by 50% compared with control-siRNA (Fig. 5A). Next, qPCR analysis from GL15 cell mRNA after CRMP5 knockdown using CRMP5-siRNA revealed 43% and 46% decrease in hes1 and hey1 mRNA levels respectively compared with control-siRNA (Fig. 5B). These results indicate that CRMP5 controls Notch downstream target, the hes1 promoter.

We then tested whether this effect could be recapitulated at the protein level. CRMP5 depletion in GL15 and A172 cells resulted in loss of Hes1 protein expression (Fig. 5C). In contrast, CRMP5 overexpression increased Hes1 protein expression in both cell lines (Fig. 5D). These experiments indicate that CRMP5 can regulate Notch signaling pathway activation in vitro as evidenced by CRMP5-dependent Hes1 expression.

In order to evaluate in vivo the consistency of the above in vitro results, we compared Hes1 protein expression levels in CRMP5HIGH and CRMP5LOW GBM tumors. First, by Western blot analysis, we were able to detect Hes1 expression only in CRMP5HIGH tumor biopsies, not in CRMP5LOW GBMs. Similarly Hes1 expression was higher in CRMP5 positive xenografts GBM_SC1 than in CRMP5 negative GBM_SC2 (Fig. 5E). Second, by immunohistochemistry, we found that Hes1 protein expression levels in GBM tumor cell nuclei paralleled CRMP5 expression (Fig. 5F). The proportion of Hes1-stained nuclei in CRMP5HIGH GBM tumors was approximately 2-fold lower than that in CRMP5LOW GBM tumors (P < 0.0001 t test; Fig. 5G). To further study the direct correlation between CRMP5 and Hes1 protein expression in human GBM, we performed a costaining using an immunofluorescence technique. We observed a coexpression of Hes1 and CRMP5 at the cellular level in CRMP5HIGH GBMs while none of CRMP5-negative cells expressed Hes1 in CRMP5LOW GBMs (Fig. 5H). These data confirm that Notch signaling pathway activation in GBM tumors is dependent on CRMP5 expression.

CRMP5 regulates lysosomal degradation of Notch1 and Notch2

To further explore the loss of Notch receptor expression under CRMP5 depletion, we first performed qPCR analysis to assess
notch1 and notch2 mRNA synthesis following CRMP5 silencing. We observed that CRMP5 depletion did not interfere with notch mRNA levels (Fig. 6A). This result suggests that CRMP5 controls Notch receptors expression at a posttranslational level. Attenuation of Notch signaling may account for Itch-dependent lysosomal degradation of the Notch receptors (39).

To explore the effect of CRMP5 on Notch protein proteolysis, we used the proteasome (MG132) or lysosome (Bafilomycin A1) inhibitor to assess Notch receptor degradation after CRMP5 knockdown. The inhibitors had no effect on cells transfected with control-siRNA, with no degradation of Notch receptors (Fig. 6B and C). When CRMP5 was depleted in GL15 or in A172 cells, application of the proteasome inhibitor MG132 did not affect Notch1 or Notch2 degradation. In contrast, lysosome inhibitor treatment of CRMP5-depleted GL15 or A172 cells counteracted Notch1 and Notch2 receptor degradation (Fig. 6B and C). These results demonstrate that CRMP5 regulates Notch1 and Notch2 lysosomal degradation, a step controlled by the E3 ubiquitin ligase Itch (39). To confirm that effects of CRMP5 on Akt activation and Notch-dependent proliferation were controlled by Itch-dependent Notch degradation, we bypassed the Notch degradation mechanism via Itch knockdown. Itch silencing had no effect on Akt activation; however, codepletion of both CRMP5 and Itch rescued CRMP5-dependent Akt activation, Notch receptor expression and Hes1 levels in GL15 and in A172 cells (Fig. 6D). Notch signaling activation in the absence of both Itch and CRMP5 indicated that Notch was no longer targeted for lysosomal degradation, and CRMP5 might protect Notch receptors from Itch-mediated lysosomal degradation.
At a functional level, we tested whether CRMP5-dependent cell proliferation loss under CRMP5 knockdown could be restored by codepletion of both CRMP5 and Itch in both cell lines. Neither GL15 nor A172 cell proliferation was influenced by Itch knockdown alone. Conversely, knockdown of both CRMP5 and Itch rescued the CRMP5-dependent loss of proliferation previously observed (Fig. 3B) to basal levels in both cell lines (Fig. 6E and F).

These data show that the loss of proliferation after CRMP5 knockdown is dependent on Itch-mediated lysosomal degradation of the Notch receptor. Accordingly, a fully functional Notch signaling pathway was rescued after silencing of both CRMP5 and Itch, leading to sustained Notch signaling activation and GBM cell proliferation.

**Discussion**

Our studies on human GBM biopsies and GBM cell lines have shown that CRMP5 is associated with a poorer prognosis and promotes cell proliferation in GBM. We characterized CRMP5 as a new player in Notch–Akt signaling, controlling Notch and Akt activation. We uncovered a novel mechanism by which CRMP5 controls Notch signaling pathway and Akt activation protecting Notch receptors from Itch-induced lysosomal degradation, leading to sustained Notch signaling activation and GBM cell proliferation.

CRMP5 controls Notch-dependent GBM proliferation *in vitro* and *in vivo*

We demonstrated a significant correlation between the mitotic index and CRMP5 expression in GBM biopsies and that CRMP5 positively modulates human GBM proliferation in GBM cell lines. Surprisingly, we previously reported an increase in neural cell proliferation in brain neurogenesis zones of adult CRMP5-deficient mice (11). This discrepancy may indicate a different role for CRMP5 in cancer versus neural cells. The increased proliferation index observed in neuroblastoma cells after CRMP5 overexpression supports this hypothesis (15). CRMP5 mRNA expression using human GBM transcriptomic analysis gave contradictory results. Using GBM TCGA data including 173 GBM, we could
not measure any difference between patient survival and CRMP1-5 mRNA expression (Supplementary Fig. S2A–S2C). However, in a cohort of 20 patients, CRMP5 emerged in a cluster of genes whose overexpression resulted in shorter patient survival (40). These contradictory observations may result from the strong expression of CRMP5 observed in glial reactive cells unrelated to tumor cells found around any brain lesions (Supplementary Fig. S2G and S2H). The careful selection of the tumor component required is more feasible in a mono-centric setting than a multicentric study. Our results on 183 GBM biopsies show that CRMP5 protein expression is related to a poorer prognosis and parallels Notch expression. Cell proliferation and cell-cycle progression are regulated by cyclin proteins. In GBM, we demonstrated that CRMP5 expression is increased in glioblastoma multiforme tissue samples in vivo, and that CRMP5 knockdown in GBM cells results in a G0/G1 phase cell-cycle arrest concomitant with a reduction in cyclin D1 protein, and an increase in Hes1 and Hey1, which are markers of the canonical Notch pathway at the transcriptional and activity levels. Accordingly, Hes1 protein expression was increased significantly in CRMP5HIGH GBM specimens compared with CRMP5LOW and in SC1stem cells. Residual Hes1 expression in CRMP5LOW GBM could be due to noncanonical activation of the hes1 promoter through other pathways such as the NF-kB pathway (46), which has been implicated in GBM pathogenesis. These results show a correlation between CRMP5 and Notch1 expression in GBM and Notch pathway activation. In addition, CRMP5 protein provides a possible mechanistic link between the intermingled pathways combining Notch and Akt activations in GBM tumors.

CRMP5 expression controls Notch receptor degradation. A, mRNA analysis of Notch1 and Notch2 mRNA after CRMP5 knockdown in GL15 cells; ywhae was used as a housekeeping gene (n = 3; Kruskal–Wallis test). B and C, Notch receptor expression under proteasome or lysosome inhibition and CRMP5 silencing in GL15 cells (B) and A172 cells (C). Actin was used as a loading control. D, Notch receptor expression and Akt activation analyzed by Western blot analysis in GL15 and A172 cells after CRMP5 and/or Itch silencing. E and F, proliferation analysis after CRMP5 and/or Itch knockdown monitored by EdU incorporation in GL15 cells (E) and A172 cells (F; n = 3; Mann–Whitney test).

Figure 6.

CRMP5 expression controls Notch receptor degradation. A, mRNA analysis of Notch1 and Notch2 mRNA after CRMP5 knockdown in GL15 cells; ywhae was used as a housekeeping gene (n = 3; Kruskal–Wallis test). B and C, Notch receptor expression under proteasome or lysosome inhibition and CRMP5 silencing in GL15 cells (B) and A172 cells (C). Actin was used as a loading control. D, Notch receptor expression and Akt activation analyzed by Western blot analysis in GL15 and A172 cells after CRMP5 and/or Itch silencing. E and F, proliferation analysis after CRMP5 and/or Itch knockdown monitored by EdU incorporation in GL15 cells (E) and A172 cells (F; n = 3; Mann–Whitney test).
activation to support sustained GBM cells proliferation and survival.

CRMP5 and Itch control Notch lysosomal degradation
Many studies have suggested that both entry and trafficking of Notch within the endocytic pathway are important in regulation of its activity (47). Notch trafficking after the early steps of Notch endocytosis requires the E3 ubiquitin ligase AIP4/Itch allowing its final targeting to the lysosome for degradation (39, 48). We demonstrated that Notch1 and Notch2 receptors are targeted for lysosomal degradation in GBM cells in agreement with other studies on mammalian cells (49). Although Itch is required for Notch receptors degradation, we demonstrated in GBM cells that CRMP5 controls Notch receptor activation. Knockdown of both CRMP5 and Itch rescued the CRMP5-dependent loss of Notch receptor expression, Akt activation and Hes1 protein as well as GBM proliferation suggesting that CRMP5 can be part of Notch receptors activation mechanism (Supplementary Fig. S7). Interestingly, few studies have shown that Notch signaling drives short-period oscillatory expression of Hes called ultradian oscillations (50). CRMP5 could be related to the control of Notch signaling ultradian oscillations by periodically protecting Notch receptors from degradation. In GBM, this control could be lost because of a pathologic event engaging CRMP5 in the protection of the Notch receptors exclusively, such as the aberrant posttranslational modification described for CRMP2 (51).

In light of these data, we present CRMP5 as a novel major mediator of Notch and Akt signaling activations that function by controlling Notch receptor lysosomal degradation. Furthermore, CRMP5 protein provides a nexus between Notch and Akt activation, contributing to a proliferative and aggressive signature in GBM and serving as an indicator of poor survival. We propose that CRMP5-dependent Notch receptor recovery could be a key oncogenic event in subsets of GBMs and that pharmacologic inhibition of CRMP5-dependent Notch receptor recovery could be a key oncotherapy, contributing to a proliferative and aggressive signature in GBM and serving as an indicator of poor survival. We propose that CRMP5-dependent loss of Notch receptor expression, Akt activation and Hes1 protein as well as GBM proliferation suggest a role for CRMP5 in GBM (39, 48). We demonstrated that Notch1 and Notch2 receptors are targeted for lysosomal degradation in GBM cells in agreement with other studies on mammalian cells (49). Although Itch is required for Notch receptors degradation, we demonstrated in GBM cells that CRMP5 controls Notch receptor activation. Knockdown of both CRMP5 and Itch rescued the CRMP5-dependent loss of Notch receptor expression, Akt activation and Hes1 protein as well as GBM proliferation suggesting that CRMP5 can be part of Notch receptors activation mechanism (Supplementary Fig. S7). Interestingly, few studies have shown that Notch signaling drives short-period oscillatory expression of Hes called ultradian oscillations (50). CRMP5 could be related to the control of Notch signaling ultradian oscillations by periodically protecting Notch receptors from degradation. In GBM, this control could be lost because of a pathologic event engaging CRMP5 in the protection of the Notch receptors exclusively, such as the aberrant posttranslational modification described for CRMP2 (51).

In light of these data, we present CRMP5 as a novel major mediator of Notch and Akt signaling activations that function by controlling Notch receptor lysosomal degradation. Furthermore, CRMP5 protein provides a nexus between Notch and Akt activation, contributing to a proliferative and aggressive signature in GBM and serving as an indicator of poor survival. We propose that CRMP5-dependent Notch receptor recovery could be a key oncogenic event in subsets of GBMs and that pharmacologic inhibition of CRMP5 could represent a promising therapeutic approach combined with Notch inhibitors.

References
CRMP5 Controls Glioblastoma Cell Proliferation and Survival through Notch-Dependent Signaling

Aubin Moutal, Jérôme Honnorat, Patrick Massoma, et al.


Updated version
Access the most recent version of this article at:
doi:10.1158/0008-5472.CAN-14-0631

Supplementary Material
Access the most recent supplemental material at:
http://cancerres.aacrjournals.org/content/suppl/2015/09/10/0008-5472.CAN-14-0631.DC1

Cited articles
This article cites 49 articles, 20 of which you can access for free at:
http://cancerres.aacrjournals.org/content/75/17/3519.full.html#ref-list-1

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.