Identification of ASF/SF2 as a Critical, Allele-Specific Effector of the Cyclin D1b Oncogene

Nicholas A. Olshavsky1,3, Clay E.S. Comstock2,3, Matthew J. Schiewer2,3, Michael A. Augello2,3, Terry Hyslop2,5, Claudio Sette6, Jinsong Zhang1, Linda M. Parysek1, and Karen E. Knudsen2,3,4

Abstract

The cyclin D1b oncogene arises from alternative splicing of the CCND1 transcript, and harbors markedly enhanced oncogenic functions not shared by full-length cyclin D1 (cyclin D1a). Recent studies showed that cyclin D1b is selectively induced in a subset of tissues as a function of tumorigenesis; however, the underlying mechanism(s) that control tumor-specific cyclin D1b induction remain unsolved. Here, we identify the RNA-binding protein ASF/SF2 as a critical, allele-specific, disease-relevant effector of cyclin D1b production. Initially, it was observed that SF2 associates with cyclin D1b mRNA (transcript-b) in minigene analyses and with endogenous transcript in prostate cancer (PCa) cells. SF2 association was altered by the CCND1 G/A870 polymorphism, which resides in the splice donor site controlling transcript-b production. This finding was significant, as the A870 allele promotes cyclin D1b in benign prostate tissue, but in primary PCa, cyclin D1b production is independent of A870 status. Data herein provide a basis for this disparity, as tumor-associated induction of SF2 predominantly results in binding to and accumulation of G870-derived transcript-b. Finally, the relevance of SF2 function was established, as SF2 strongly correlated with cyclin D1b (but not cyclin D1a) in human PCa. Together, these studies identify a novel mechanism by which cyclin D1b is induced in cancer, and reveal significant evidence of a factor that cooperates with a risk-associated polymorphism to alter cyclin D1 isoform production. Identification of SF2 as a disease-relevant effector of cyclin D1b provides a basis for future studies designed to suppress the oncogenic alternative splicing event. Cancer Res; 70(10); 3975–84. ©2010 AACR.

Introduction

The cyclin D1b variant, produced via alternative splicing of the CCND1 transcript (1, 2), is a potent oncogene that harbors distinct functions from full-length cyclin D1 (cyclin D1a; refs. 3–5). Unlike cyclin D1a, cyclin D1b independently confers cellular transformation (4, 5). In addition, only the cyclin D1b isoform has the capacity to promote anchorage-independent growth and cell invasiveness (6). Further confirmation of novel oncogenic capabilities was identified in mouse models, wherein animals expressing human cyclin D1b under the bovine K5 promoter showed increased papilloma multiplicity (3). Given the enhanced oncogenic function of cyclin D1b, it is imperative to define the mechanisms that regulate cyclin D1b production in systems of clinical relevance.

The alternative splicing event that produces the cyclin D1b transcript (referred to as transcript-b) arises from failure to splice at the CCND1 exon 4–intron 4 boundary. Due to intronic transcriptional termination, cyclin D1b lacks exon 5–encoded sequences and contains a novel COOH-terminal domain of unknown function (1, 2). Previous studies showed that transcript-b/transcript-a ratios are enhanced in selected tumor types, thus providing evidence that the alternative splicing event may be altered as a function of tumorigenesis or tumor progression (6–10). Lending support to this posit, recent analyses of a large cohort of prostate cancer (PCa) specimens revealed that cyclin D1b (but not cyclin D1a) is induced in PCa as compared with nonneoplastic tissue (11). These findings were of interest, as cyclin D1b has specialized functions in this tumor type that are hypothesized to promote tumor progression (7).

Despite the compelling evidence identifying cyclin D1b as a potent, novel oncogene, the tumor-associated factor(s) that promote the alternative splicing event remain poorly defined. It has long been suggested that a polymorphism within the exon 4 splice donor site (G/A870) might contribute to transcript-b production, wherein the A allele was suggested to favor the alternative splicing event (1, 12, 13). Recent analysis using minigenes supported this contention,
and analysis of nonneoplastic prostate tissue showed that the presence of the A allele predicted for higher transcript-b production; however, the effect of the A allele was lost in tumor tissue, thus indicating that tumor-associated factor(s) likely bypass or modify the effect of the G/A870 polymorphism with regard to transcript-b production (11). Here, the present study identifies the SF2 (also known as ASF or SRp30a) RNA binding protein as a critical, allele-selective factor that associates directly with transcript-b and modulates cyclin D1b production in model systems of cancer relevance. Importantly, analyses of tumor tissue further support a model wherein tumor-associated elevation of SF2 specifically enhances cyclin D1b expression in human disease. Together, these findings provide a mechanism by which CCND1 alternative splicing is controlled in tumorigenesis, and identify SF2 as a critical regulator of cyclin D1b oncogene production.

Materials and Methods

Cell culture, transfections, generation of stables. LNCaP, C33A, and LAPC4 cell lines were obtained, cultured, and transfected as previously described (7, 14–17). The DT40-ASF cell line was a generous gift from James Manley (Cell and Molecular Biology, Columbia University, New York, NY) and maintained as previously described (18). DT40-ASF cells were transfected using the Amaxa Nucleofector protocol. C33A stable cell lines were generated by transfecting the indicated expression constructs encoding empty vector, or individual cyclin D1 minigenes and selected with 400 μg/mL of G418 (MP Biomedicals). Clonal isolates were screened for expression by immunoblot. Isolates used herein are denoted as C33A-Vec, C33A-G1 (containing the G870 allele minigene), and C33A-A1 (containing the A870 allele minigene).

Plasmids. The pCGT7, pCGT7-ASF/SF2, and pCGT7-SRp40 expression constructs were generous gifts from Adrian Krainer (Cell Biology and Signal Transduction, Institute of Biomedical Sciences, Taipei, Taiwan) and was previously described (20). Given the enhanced oncogenic function of the cyclin D1b variant (3–5), and the established observation that cyclin D1b levels are enhanced as a function of prostate tumorigenesis (7, 11), it is imperative to discern the mechanism(s) underpinning the alternative splicing event. As was recently reported, the CCND1 G/A870 polymorphism plays a context-specific role in the alternative splicing event, wherein the A allele favors cyclin D1b production in minigene expression studies in which the alleles were individually examined (11). These studies were validated here, wherein minigenes harboring either the G or A870 allele (Fig. 1A) were independently introduced by stable transfection into C33A cells, which have been previously shown to harbor low to undetectable levels of endogenous cyclin D1 isoforms (24). Introduction of the G allele minigene (stable line C33A-G1), resulted in detectable expression of both CCND1 transcripts (a and b), but a preference towards transcript-a and cyclin D1a protein was observed (lane 2). Transcript-b levels were comparatively increased in the presence of the A allele minigene (lane 3), consistent with previous studies in which the minigenes were individually analyzed in parallel (11).
Subsequent analyses of individual minigene studies in spontaneously immortalized CV1 cells or viral oncoprotein immortalized RWPE-1 prostate epithelial cells (25, 26) were also carried out. Similar findings were observed in both nontumorigenic model systems, in which the A allele predisposed to transcript-b and cyclin D1b production (Supplementary Fig. S1). These studies further support the premise that in isolated comparisons, the G/A870 polymorphism influences CCND1 alternative splicing.

As G/A870 lies within the splice donor site, it was reasoned that the polymorphism might influence RNA binding protein recognition or activity. The splicing factor binding resource ESEfinder3.1 (27, 28) was therefore used to predict distinctions in the profile of associated splicing factors between the G and A-870 allele splice donor sites. As shown, binding of SF2 was suggested to be altered in both position and strength by changes in the polymorphic site (Fig. 1C).

To initially assess whether SF2 could play a role in CCND1 alternative splicing, RIP was performed using the same stable cell lines described in Fig. 1B. Using standard techniques, RNA associated with either SF2-specific or control antisera were isolated, reverse transcribed, and detected by PCR amplification. As illustrated in Fig. 1D (top), transcript-b was only weakly detected in control cells and immunoprecipitates thereof (lanes 1–3). In cells containing the G allele minigene (C33A-G1), SF2 readily associated with transcript-b, providing the first evidence that SF2 associates with this mRNA species (lanes 4–6). SF2 association with transcript-b was reduced in cells containing the A allele minigene (lanes 7–9), as was confirmed by quantitative reverse transcription-PCR (Fig. 1D, bottom). For these analyses, signals observed in C33A-VeC cells were set to “1” for ease of comparison. The finding that SF2 binding is influenced by the polymorphism was subsequently confirmed in additional stable isolates of minigene integration (Supplementary Fig. S2A) as well as through transient analyses (B). Together, these data strongly indicate, for the first time, that the G/A870 polymorphism influences recognition by the SF2 splicing factor.

**SF2 specifically correlates with cyclin D1b in prostate cancer.** The observation that the G/A870 polymorphism may alter SF2 association with transcript-b was of interest, given reports that cyclin D1b, not cyclin D1a, is induced as a function of prostate tumorigenesis (7, 11), and that cyclin D1b has oncogenic activity (3–5). Intriguingly, analyses of gene microarray data from human PCa showed that SF2 expression increases with tumor progression (Fig. 2A; refs.
29–31). This observation was further validated upon screening a panel of PCa cell lines, wherein it was revealed that SF2 expression is lowest in nontransformed RWPE-1, and as compared with cells derived from primary and distant metastases of prostate cancer (Supplementary Fig. S3). Among the cancer cell lines representative of hormone therapy-sensitive disease, SF2 was relatively low in cells known to express lower levels of cyclin D1b (LNCaP) and higher in cells of this subtype known to express high levels of cyclin D1 (LAPC4; refs. 7, 11). For the androgen receptor-negative cells, unexpectedly high levels of expression were observed in PC3 cells, which exhibit the most aggressive phenotype in vivo with regard to metastases (32). To probe the effect of SF2 on cyclin D1b production in human tumors, comparative analyses of nuclear SF2 and cyclin D1 (a and b isoforms) expression was objectively analyzed using serial sections of PCa specimens and quantitative AQUA analyses. Representative images of tumors scoring with low SF2 (Fig. 2B, top) or high SF2 (bottom) are shown. Signals were quantified across each specimen within the nuclear compartment (DAPI positive, blue) of epithelially derived carcinoma cells (cytokeratin positive, green), and resultant data are plotted in Fig. 2C. As shown, tumors expressing low SF2 exhibited low cyclin D1b expression; conversely, tumors with high SF2 scored high for cyclin D1b. Quantification of all tumors examined revealed a significant correlation between SF2 and cyclin D1b (P = 0.023), but not cyclin D1a (P = 0.19). Coexpression of cyclin D1b and SF2, in serial sections, was observed in glandular epithelial cells (data not shown). Together, these data show that SF2 expression is strongly correlated with only the cyclin D1b isoform in human disease.
SF2 modulates cyclin D1b production in multiple model systems. Because SF2 predicted for high cyclin D1b in PCa, the effect of SF2 on cyclin D1b production was examined in model systems of disease relevance. Initially, LNCaP cells, which express low endogenous levels of cyclin D1b, were used (7). Epitope-tagged SR proteins (SRp40 or SF2) were individually introduced (Supplementary Fig. S4) by transient assays with high transfection efficiency (Supplementary Fig. S5), and the effect on CCND1 mRNA isoform expression determined (Fig. 3A). Consistent with previous results, these cells express low levels of endogenous transcript-b (lane 1), and SRp40 failed to elicit changes in either CCND1 transcript (lane 2). Significantly, SF2 promoted a dramatic increase in transcript-b expression without altering transcript-a (lane 3). Quantification revealed a ~2.7-fold increase in transcript-b following SF2 introduction (right), thus providing the first functional evidence for SF2-mediated regulation of cyclin D1b. Increased expression of SF2 in LNCaP cells also resulted in an increase in cyclin D1b protein expression (Supplementary Fig. S4). Unfortunately, depletion of endogenous SF2 in PCa models using short interfering RNA were unsuccessful (data not shown), consistent with previous observations that knockdown of SF2 induces cell death (33). As such, a previously developed DT40-ASF/SF2 cell line was used (DT40-ASF), in which endogenous SF2 was replaced with a tet-repressible SF2 construct (Fig. 3B; ref. 18). In this system, SF2 expression was undetectable ~24 hours following the addition of doxycycline. Previous reports indicated that 48 hours post-knockdown, the DT40-ASF cell line begins to undergo apoptosis (33). Thus, experiments analyzed end-points after 24 hours, whereupon no loss of cell viability was noted (data not shown). As expected, no detectable cyclin D1 isoforms were recognized (Fig. 3B, left, lanes 1 and 2). Stable introduction of the G870 minigene revealed that although both isoforms were produced (left, lanes 4 and 5), suppression of endogenous SF2 (left, lane 5) diminished cyclin D1b (but not cyclin D1a) production. Similar results were observed at the level of the transcripts following depletion of SF2 (Fig. 3B, right). Collectively, these results identify SF2 as an effecter of cyclin D1b production in multiple model systems.

Endogenous SF2 associates with both alleles in PCa cells. As it was observed that elevated SF2 induces cyclin D1b in PCa (Fig. 2), and that SF2 differentially binds the transcript dependent on G/A870 (Fig. 1), the effect of the polymorphism on SF2 association was investigated in PCa cells. First, LAPC4 cells were used, which are homozygous for the A870 allele (7). Consistent with the individual minigene analyses in Fig. 1, endogenous SF2 associates with the endogenous transcript-b in these cells (Fig. 4A). RFLP analyses of the SF2-associated transcript was also performed (Fig. 4B). These observations show that in PCa, SF2 can associate with transcript-b generated by the A870 allele; however, as individual minigene analyses in Fig. 1 indicated a preference for SF2 to associate with transcript-b from the G870 allele (when compared in isolation), the most critical analyses were generated in LNCaP cells, which are heterozygous for the polymorphism (shown as genotype control in Fig. 4B). This model system afforded the first opportunity to dissect the effect of SF2 on G870-derived versus A870-derived transcripts in the same model system. As expected, SF2 was associated with total transcript-b in this model system (Fig. 4C). Using the SF2-bound and recovered transcript, two key determinations were made. First, RFLP analyses of the total transcript (Fig. 4D, lane 1) revealed that ~61% of the endogenous transcript-b was derived from the G allele and 39% from the A allele, thus indicating that in the endogenous setting, both alleles significantly contribute to transcript-b production. Intriguingly, RFLP analyses and
quantification of the SF2-bound fraction (Fig. 4D, lane 2) resulted in a similar ratio of transcript generated from the G and A alleles, based on at least five independent analyses and quantification (Fig. 4D, bottom). Thus, these data show that endogenous SF2 could associate with both transcripts when present, but shows a slight preference for the G870-derived transcript.

**SF2 predominantly associates with and produces transcript-b from the G allele.** Given the observation that elevated SF2 correlates with high cyclin D1b in human PCa and is sufficient in PCa model systems to induce cyclin D1b production, the effect of the G/A870 polymorphism for this event was determined. As shown, SF2 levels were elevated through ectopic expression of an HA-tagged allele in cells heterozygous for the polymorphism and that express low endogenous cyclin D1b (Supplementary Fig. S6), so as to mimic tumor-associated SF2 induction. Subsequent analyses (Fig. 5A) confirmed SF2-mediated enhancement of overall transcript-b levels (compare lanes 1 and 4). In addition, SF2 association with transcript-b was markedly enhanced in the SF2-transfected cells (compare lanes 2 and 5), demonstrating that elevated SF2 results not only in enhanced transcript-b production, but also enhanced SF2-associated transcript.

Furthermore, these findings also allowed determination of (a) the allele composition of transcript-b levels elevated by SF2 and (b) whether elevated SF2 altered the likelihood of either allele to directly associate with SF2. To answer both questions, RFLP analyses were performed with the SF2-associated transcript.
transcripts after SF2 induction or in control cells. First, it was observed that SF2 predominantly resulted in an induction of transcript-b from the G870 allele (Fig. 5B, lane 1 versus lane 3; quantified in C). These data implicate SF2 as an allele-selective modifier of the alternative splicing event. Second, to determine whether this action of SF2 was direct, RFLP analyses of the SF2-bound fraction was compared in cells with steady-state SF2 (control) versus those with elevated SF2 expression (Fig. 5B, compare lanes 2 and 4). For ease of comparison, the percentage of SF2 bound to the A870-derived transcript (relative to input) in control cells was set to “1”, and relative association determined for each (Table 1). As shown, SF2 association with the G allele was statistically unchanged under conditions mimicking tumor-associated SF2 elevation. By contrast, SF2 association with the A allele was enhanced by ~2-fold under these conditions.

Collectively, these data suggest a model (summarized in Fig. 6) in which SF2 elicits allele-selective effects on cyclin D1b production in prostate cancer that are dose-dependent. Under steady state conditions, SF2 binds with slight preference to transcripts generated from the G870 allele; concordantly, the G allele accounts for 61% of transcript-b production with the A allele accounting for the remaining 39%. These data are consistent with the supposition that SF2 acts (potentially directly) to suppress splicing at the intron 4–exon 4 boundary, and that this event is facilitated by the G870 allele. Elevated SF2, such as observed in human disease, can modestly enhance SF2 association with the A allele transcript, but induced SF2 remains predominantly associated with and produces cyclin D1b from the G allele transcript.

**Discussion**

Here, we identify SF2 as a dose-dependent effector of cyclin D1b production, and provide evidence of a tumor-associated mechanism that alters the influence of the G/A870 polymorphism. Although minigene analyses of individual alleles (Fig. 1) and evaluation of nonneoplastic human tissue support a role for the A870 allele in promoting transcript-b and resultant cyclin D1b production, the influence of the A allele is thought to be relieved in PCa (11). The present data shows that the RNA-binding protein SF2, which is induced as a function of PCa progression, predicts for cyclin D1b (not cyclin D1a) elevation in human disease (Fig. 2). By contrast, cyclin D1b production was attenuated in model systems of SF2 depletion (Fig. 3). Functional studies in PCa cells heterozygous for the polymorphism unexpectedly showed that SF2 predominantly associates with and induces transcript-b derived from the G870 allele; however, association with and production of transcript-b from the A allele could still occur (Figs. 4 and 5). Together, these studies provide a novel mechanism by which cyclin D1b oncogene production is induced in human disease, and identify tumor-associated SF2 as an allele-selective effector of cyclin D1b.

Despite the potent oncogenic activity of cyclin D1b, knowledge of the factor(s) that regulate the CCND1 alternative splicing event are poorly defined. The present identification of SF2 as an effector of transcript-b and cyclin D1b production using both in vitro models and analyses of human tumors provides strong evidence linking splicing factor deregulation to oncogene activation. It is tempting to speculate as to whether SF2 could cooperate with few effectors of cyclin D1b production that were identified in other tissue types. For example, in colorectal cells, it was observed that knockdown of the SWI/SNF chromatin remodeling complex subunit Brahma (BRM) increased transcript-b production without altering transcript-a (34). These findings are of note.

**Table 1. Relative SF2 association**

<table>
<thead>
<tr>
<th>% Allele</th>
<th>Control</th>
<th>SF2</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.0</td>
<td>1.92 ± 0.57</td>
</tr>
<tr>
<td>G</td>
<td>5.34 ± 0.80</td>
<td>4.42 ± 0.80</td>
</tr>
</tbody>
</table>

**Figure 5.** Elevated expression of SF2 predominately associates with and promotes transcript-b production from the G allele. A, LNCaP cells were transfected with expression constructs encoding empty vector or HA-SF2. Forty-eight hours later, cells were harvested and subjected to a RIP. Transcript-b expression was analyzed as previously described (Fig. 1). B, input and SF2-associated RNA from A were then subjected to RFLP analyses as in Fig. 4D. Representative analyses are shown with their respective ratios of transcript-b to A to G (A/G). C, to assess the effect of SF2 elevation on allele-specific transcript-b production, relative change in transcript abundance generated from the G or A allele after SF2 introduction was determined by quantification of the “input” RFLP signal (represented by B, percentage of change in lane 3 versus lane 1) from four independent experiments. Average change in relative G or A allele derived transcript per experiment was averaged and plotted as the percentage of D1b change over vector control.
as BRM was recently shown to be significantly downregulated in human PCa (35), and it has yet to be determined whether BRM loss might also affect SF2 levels. In a separate study, a chromosomal translocation-derived transcription factor known to be upregulated in Ewing’s sarcoma (EWS-FLI1) was found to enhance cyclin D1b production by diminishing the rate of transcriptional elongation (10). More recently, it was shown that Sam68 promotes cyclin D1b production through a splicing-repressive mechanism by blocking U1-70k association, a constitutive spliceosome accessory factor of the U1 small nuclear ribonucleoprotein that is necessary for 5’ splice site recognition (36, 37). It is well established that chromatin remodeling complexes could alter RNA Pol II accessibility by alteration of the native chromatin structure (38); moreover, pre-mRNA splicing occurs cotranscriptionally, and is aided by the function of SR proteins (including SF2) which can bind to Pol II and selected SWI/SNF subunits (BAF155 and BAF53A; ref. 39). Therefore, an attractive hypothesis is that SF2 could act in concert with either BRM or EWS-FLI1 to modulate the CCND1 splicing event. Given the marked protumorigenic activity of cyclin D1b, these collective observations further underscore the importance of delineating the mechanisms that regulate or influence the SF2-mediated alternative splicing event in models of disease relevance.

With regard to clinical relevance, it is notable that in PCa cells, SF2 exhibited an allele-selective effect on the alternative splicing event. Previous studies showed that in nonneoplastic tissue, the A870 allele was associated with higher transcript-b production but that the influence of the A allele was lost in PCa specimens (11); these findings suggested that tumor-associated factors might either bypass the effect of the polymorphism or bolster the production of transcript-b from the G allele. The present data supports the hypothesis that SF2 may serve as such a factor because SF2 induction (such as occurs in human disease) predominantly binds to transcript-b derived from the G allele and promotes the accumulation of this transcript. Production of transcript-b still occurs from the A allele (~40%), however, this seems to remain unaltered following modulation of SF2 expression, thus it will be of interest to examine the functional relationship between SF2 and the A allele. Examination of how SF2 influences allele-specific cyclin D1b production in other tumor types in which cyclin D1b levels are elevated as a function of tumorigenesis (e.g., colon, bladder, or breast carcinoma) will be critical (6, 9, 40, 41). Given the propensity of SF2 to preferentially bind to and induce cyclin D1b from the G allele, the present data indicate that SF2 may promote intron inclusion at the CCND1 exon 4–intron 4 boundary, and preclude for a splicing repressor function of SF2 was previously established for the MNK2 kinase (42). It cannot be presently ruled out that the effect of SF2 could be manifested through other means, given the ability of SF2 to affect mRNA metabolism, mRNA transport and/or stability, and mTOR-mediated translation (43, 44). Future analyses will be directed at defining the action of SF2 at the exon 4–intron 4 boundary.

Finally, the present findings provide new insight into a potential means through which SF2 promotes cellular transformation. It is noteworthy that SF2 could independently induce transformation and induce tumor growth in vivo (42), thus demonstrating phenotypes similar to those observed with cyclin D1b (3, 5). As SF2 levels correlated with cyclin D1b in human PCa, these data implicate cyclin D1b as a possible downstream effector of SF2-mediated cellular transformation in the prostate. Additional mechanisms are predicted to contribute to this event, as perturbations in spliceosome function and RNA processing proteins have been recently identified as major contributors to genomic instability (45). Importantly, dysregulation of splicing factors has also been shown to accelerate PCa progression and metastases, such as observed by deregulated expression of SRp40, or in the presence of specific, cancer risk–associated polymorphisms in the intronic region of the KLF6 tumor suppressor that create novel SRp40 binding sites (46). Given the disease relevance of this event, it will be imperative to determine not only the consequence of SR protein dysregulation, but whether these events act in concert to promote tumor progression. Ongoing studies suggest that tumor-associated SF2 induction
may induce and enhance the migration and invasion phenotype in prostate cancer cells (data not shown), providing the impetus to discern the effect of SF2 on the development of tumor metastases.

In summary, our study identifies SF2 as a novel, clinically relevant effector of CCND1 alternative splicing, capable of promoting allele-specific induction of cyclin D1b in prostate cancer. The findings presented are among the first to determine how the cyclin D1b oncogene is enhanced in human disease, and provide the foundation for future studies directed at developing mechanisms to target oncogene induction.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

13. Howe D, Lynas C. The cyclin D1 alternative transcripts [a] and [b] are expressed in normal and malignant lymphocytes and their relative levels are influenced by the polymorphism at codon 241. Haematologica 2001;86:563–9.

Acknowledgments

We thank the K. Knudsen lab for ongoing discussions, Dr. E. Knudsen for critical commentary, Drs. J. Bruzik, C. Chalfant, and R. Carstens for valuable discussions, and J. Tulenko for assistance with figure presentation.

Grant Support

NIH grants RO1 CA09996 and CA116777 (K.E. Knudsen), Associazione Italiana Ricerca sul Cancro and Association for International Cancer Research (C. Sette), and a DOD New Investigator Award (C.E.S. Comstock). M.J. Schiewer and M.A. Augello were supported by predoctoral fellowships from the Department of Defense (PC094195 to M.J. Schiewer and PC094596 to M.A. Augello), and N.A. Olshavsky is supported by NIEHS training grant 2T32ES007250-21A1.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received 09/21/2009; revised 03/10/2010; accepted 03/10/2010; published OnlineFirst 05/11/2010.

www.aacjrournals.org Cancer Res; 70(10) May 15, 2010 3983


Identification of ASF/SF2 as a Critical, Allele-Specific Effector of the Cyclin D1b Oncogene


Cancer Res 2010;70:3975-3984. Published OnlineFirst May 11, 2010.

Updated version
Access the most recent version of this article at:
doi:10.1158/0008-5472.CAN-09-3468

Supplementary Material
Access the most recent supplemental material at:
http://cancerres.aacrjournals.org/content/suppl/2010/05/11/0008-5472.CAN-09-3468.DC1

Cited articles
This article cites 45 articles, 25 of which you can access for free at:
http://cancerres.aacrjournals.org/content/70/10/3975.full.html#ref-list-1

Citing articles
This article has been cited by 12 HighWire-hosted articles. Access the articles at:
/content/70/10/3975.full.html#related-urls

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.