The Fra-1/AP-1 Oncoprotein

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Among components of the AP-1 complex, the FOS-family transcription factor Fra-1, encoded by FOSL1, has emerged as a prominent therapeutic target. Fra-1 is overexpressed in most solid tumors, in response to the BRAF-MAPK, Wnt-beta-catenin, Hippo-YAP, IL-6-Stat3, and other major oncogenic pathways. In vitro functional analyses, validated in onco-mouse models and corroborated by prognostic correlations, show that Fra-1-containing dimers control tumor growth and disease progression. Fra-1 participates in key mechanisms of cancer cell invasion, Epithelial-to-Mesenchymal Transition, and metastatic spreading, by driving the expression of EMT-inducing transcription factors, cytokines, and microRNAs.

Keywords: transcription factor ; AP-1 complex ; FOSL1

1. Introduction

The oncogenic transcription factors (TFs) were originally envisaged as ideal targets for anticancer therapies [1], and encouraging preclinical/clinical results were obtained by targeting the TFs harboring binding sites for small molecules, such as the Stat3 SH2-phosphorylation domain and the hormone-binding domains of the steroid receptors. Nevertheless, besides the well-characterized DNA-binding domains, most TFs exhibit disordered secondary structures and lack catalytic sites and binding pockets. Thus, differently from therapeutically targeted receptors and cytoplasmic protein kinases, TFs are generally considered “undruggable”.

The AP-1 complex [2][3] results from dimerization between members of the JUN (c-Jun, JunB, and JunD) and FOS (c-Fos, FosB, Fra-1, and Fra-2) families, along with other transcription factors (ATF and Maf families). These proteins share the bZIP domain, in which the DNA-contacting basic amino acid-rich region is flanked by the leucine zipper, which mediates the dimerization, resulting in the large variety of JUN/FOS homo- and hetero-dimers.

Recently, based on the machine-learning-based AlphaFold method [4], the structures of each component of the human proteome have been predicted and made available [5]. As for the other FOS proteins, the Fra-1 structure can be modeled (with high to very high confidence) only for the 70–80-aa region encompassing the DNA-binding bZIP region, while most of the protein appears intrinsically unstructured. Therefore, the inherently disordered Fra-1 regions are likely to assume defined structures following the Fra-1/AP-1 interaction with partner molecules.

Several AP-1 components are overexpressed and/or post-translationally modified in response to the major oncogenic pathways. However, various lines of evidence, including the genetic inactivation of individual *JUN* and *FOS* family members in onco-mouse models, show that individual AP-1 proteins can exhibit cell context-dependent oncogenic or tumor suppressor roles, as highlighted in a seminal review entitled: “AP-1: a double-edged sword in tumorigenesis” [6]).

2. FOSL1/Fra-1 Structure and Regulation

Among *FOS* family members, the transcription factor Fra-1 is a major driver of cancer cell invasion, EMT (Epithelial-to-Mesenchymal Transition), and metastasis (reviewed in [7][8][9]). The 271 amino acids Fra-1 protein is encoded by the *FOS*-related gene *FOSL1*, localized on chr11q13 [10].

FOSL1 is overexpressed in the aggressive variants of most solid tumors in response to a variety of extranuclear (RTKs, RAS, and BRAF) and nuclear (MYC, AP-1) oncoproteins. Stat3 and Tcf/Lef elements mediate cancer-associated *FOSL1* induction in response to the IL6 and *Wnt*-beta-catenin pathways, respectively (reviewed in [7][8][9]).

The sequential epigenetic events involved in *FOSL1* transcriptional elongation depend on both upstream and intronic enhancers, controlled by multiple nuclear oncoproteins, such as c-Myc and AP-1. The pathway responsible for the ERK-induced recruitment of c-Myc to the *FOSL1* promoter in response to neuregulin (NRG1) has been recently elucidated in breast cancer [11]. Multiple AP-1 binding sites mediate the *FOSL1* positive autoregulation, which amplifies the effect of

Fra-1 posttranslational accumulation. The enhancer-associated epigenetic reader BRD4 drives the recruitment of p-TEFb (positive-Transcription Elongation Factor-b), which phosphorylates the RNAPII (RNA polymerase II) CTD (Carboxy-Terminal-Domain), thus triggering transcriptional elongation by the release of the RNAPII paused on the *FOSL1* promoter [12]. Notably, the *FOSL1* intronic enhancer is part of a much larger SE (Super Enhancer) region, identified by genome-wide analyses in glioblastoma multiforme (GBM) [13], pancreatic, and colorectal cancer cells [14].

FOSL1 is post-transcriptionally inhibited by multiple miRNAs. Cancer-associated downregulation of miR-34a/c and miR-15/16-family member miR-497 contributes to the Fra-1-driven neoplastic cell invasion and EMT in breast and colorectal cancer [15][16][17]. Downregulation of miR-19a-3p participates in Fra-1 accumulation in TAMs (Tumor-Associated Macrophages) recruited to breast tumors microenvironment, in which the miR-19a-3p-Fra-1-Stat3 pathway controls the macrophage polarization towards the pro-neoplastic immunosuppressive M2 phenotype [18]. The regulatory mechanisms of miRNA activity include the competition for miRNA binding (sponging) performed by several classes of non-coding RNAs, including the recently characterized circular RNAs (circRNAs). Given their extraordinary stability, circRNAs represent highly effective miRNA sponges [19]. Interestingly, the Genome Browser tracks for circRNAs show the hsa_circ_0022924 [20] deriving from circularization of the *FOSL1* distal exon and including the whole *FOSL1* 3′UTR. Therefore, this cirRNA is a candidate competing for endogenous RNA (ceRNA) sponging the oncosuppressor miRNAs (miR-34 and miR-15/16 family members, along with miR-19a-3p and miR-29a) that downregulate the expression of Fra-1 and other oncoproteins.

The ubiquitin-independent turnover of Fra-1 is prevented by the phosphorylation of S252 and S265 serine residues mediated by ERK2- and Rsk1, respectively [21], and by PKC-theta-dependent phosphorylation of T223 and T230 threonine residues [22]. In turn, the Fra-1 stabilization in response to the RTK-RAS-RAF-MEK pathway indirectly controls the stability of the c-Jun heterodimeric partner [23].

Accordingly, the BRAF and MEK inhibitors decrease Fra-1 protein accumulation by directly affecting its stability and indirectly abrogating the Fra-1/AP-1-mediated transcriptional autoregulation [24]. In addition, the ubiquitin-independent Fra-1 degradation requires the Fra-1 phosphorylation-independent association with the proteasomal subunit TBP-1 to mediate the proteasomal recognition of the poorly structured Fra-1 C-terminal region [25].

3. Fra-1 in Tumor Growth, Invasion, and Metastasis

Fra-1 overexpression crucially contributes to cancer cell invasion in most solid tumors, including adenocarcinoma (breast, lung, colon, pancreas, and thyroid), squamous cell carcinomas, and non-epithelial cancers, such as melanoma, malignant mesothelioma, and GBM (reviewed in [9]).

Fra-1 drives the morphological changes in cytoskeletal organization, loss of epithelial polarization, increased motility, and invasiveness, which reflect different context-dependent degrees of mesenchymal transformation, from partial to complete EMT [7]. Accordingly, in breast and colorectal adenocarcinoma cell lines the Fra-1-dependent transcriptomes and cistromes comprise well-characterized EMT-inducers, including tyrosine kinase receptors (AXL), EMT-inducing cytokines (TGF-beta and IL-6), EMT-TFs (ZEB1 and ZEB2), and chromatin components (HMGA1) [26][27][28][29][30][31]. In addition to the EMT-related pro-invasive programs, Fra-1 target genes control cell proliferation, survival, and anoikis resistance [32][33][34][35][36][37][38], as summarized in.

Fra-1 contributes to both autocrine and paracrine mechanisms of EMT and tumor angiogenesis, by inducing multiple cytokines, including TGF-beta in breast and colorectal cancer cells [29][30], and IL-6 and VEGF in the TAMs recruited to tumor microenvironment [18][39][40][41].

Fra-1 downstream effectors also include relevant non-coding transcripts. Fra-1 controls the transcription of the broadly overexpressed onco-miRNA miR-21, which, in turn, contributes to positive feedback loops with AP-1 in RAS-transformed cancer cells [42][43][44]. Another positive feedback is mediated by the Fra-1-dependent control of miR-134 in ovarian cancer. miR-134 inhibits the Protein Phosphatase-1 (PP1) regulatory subunit SDS22, thus potentiating the ERK and JNK MAPK signaling and Fra-1 accumulation and driving cancer cell proliferation, migration, and invasion [45]. Non-coding RNAs also participate to the Fra-1 dependent control of Epithelial to Mesenchymal Transition. For example, the Fra-1-mediated induction of miR-221/222 controls the miR-221/222-TRPS1-ZEB2 pathway, which promotes EMT in breast cancer cells [46].

Fra-1 plays a pivotal role in the dynamic balance between cancer and non-cancer stem cells (CSCs). In breast cancer cells, the Twist- and Snail-mediated induction of *FOSL1* results in Fra-1 accumulation, which drives the EMT-associated transition from non-CSCs to CSCs [47]. In colorectal cancer cells, IL-6 potentiates the Fra-1 activity by inducing the

HDAC6-mediated Fra-1 deacetylation and accumulation, resulting in the gain of stem-like features, partially dependent on the Fra-1-mediated transactivation of the *NANOG* promoter [48]. In NF1-mutant GBM tumors and cell lines, *FOSL1* overexpression has been recently implicated in the control of mesenchymal subtype and gain of stem-like features. Accordingly, in a mouse model of GBM, *FOSL1* deletion drives the transition from mesenchymal to proneural transcriptional signature, along with decreased stemness and tumor growth [49]

4. Fra-1 as Prognostic Biomarker and Cancer Cell Addiction to Fra-1 Overexpression

RNA expression profiling and IHC data show the prognostic relevance of Fra-1 and/or Fra-1-dependent transcriptomes. Time to recurrence and/or metastasis-free survival correlate with Fra-1 expression (alone or in multivariate analyses) in a wide range of adenocarcinomas, including breast [29][34][36][50][51], colon [30][35][41][48], lung [37], pancreas [37], cholangiocarcinoma [52], and squamous cell carcinomas, such as HNSCC (Head and Neck Squamous Cell Carcinoma) [53][54], ESCC (Esophageal Squamous Cell Carcinoma) [55][56], and OSCC (Oral Squamous Cell Carcinoma) [57]), along with non-epithelial cancers, such as glioma [58].

Interestingly, in TNBC (Triple-Negative Breast Cancer), the gene signature (Fra-1 classifier) derived from experimentally determined Fra-1-transcriptomes exhibits predictive value superior to most breast cancer prognostic classifiers [34]. Among the therapeutically promising Fra-1-regulated genes in invasive breast cancer [34][36], *ADORA2B* renders the Fra-1-overexpressing TNBCs vulnerable to Adenosine$_{2b}$ receptor inhibitors, such as the common anti-asthmatic theophylline [34]. In multiple tumors, additional synthetic-lethal interactions involve various “druggable” proteins, encoded by Fra-1 target genes and coexpressed with *FOSL1*, including receptors (e.g., *AXL* and *PLAUR*) [28][59][60], cytokines (e.g., *IL6* and *TGFB2*) [26][29][30], and mitotic kinases (e.g., *AURKA*) [37].

The context-dependent roles of Fra-1 expression are pinpointed by the inhibitory effects of *FOSL1* downregulation on tumor growth, detectable in *KRAS*-mutated but not in *KRAS*-wild type PDAC (Pancreatic Ductal AdenoCarcinoma) and LUAD (LUng ADenocarcinoma) cells. As previously shown in RAS-transformed thyroid cells [61], Fra-1 knockdown induces G2-M arrest and apoptosis in *KRAS*-mutated LUAD cells. Accordingly, the knockdown or pharmacological inhibition of Fra-1-controlled mitotic regulators recapitulates the effects of *FOSL1* loss. In *KRAS*-mutated, but not in *KRAS*-wild type lung cancer cells, AURKA depletion selectively blocks cell proliferation and expression of mitotic regulators (*AURKA*, *CCNB1*, *HURP*, *TACC3*, and *PLK1*), though AURKA overexpression is insufficient to rescue all the effects of *FOSL1*-knockdown in *KRAS*-mutated cells [37].

Similarly, *ID1* expression is prognostically relevant in *KRAS*-wild type but not in *KRAS*-mutated LUAD. The ID1 effects on cell proliferation and mitotic machinery largely depend on the ID1-mediated control of *FOSL1*. Interestingly, *FOSL1* re-expression can rescue the *ID1*-silenced phenotype in *KRAS*-mutated cells [62].

Along with *KRAS* mutation, loss of *SMAD4* is a key event in pancreatic cancer progression and metastatic dissemination. Recently, a high-throughput screen for prometastatic SMAD4 target genes has identified *FOSL1*, which is negatively regulated by SMAD4 direct binding to the enhancer region of *FOSL1*. In turn, Fra-1 is necessary and sufficient to recapitulate the effect of SMAD4 loss on metastatic lung colonization [63].

Cancer cell addiction to Fra-1-containing dimers is strongly supported by recent unbiased CRISPR-Cas9 screens to identify dependencies in hundreds of genomically characterized cell lines representing most human cancers [64].

According to the Broad Institute Project Achilles, 205/808 cancer cell lines depend on *FOSL1* expression, while the Sanger’s Cancer Dependency Map shows addiction to *FOSL1* in 50/323 lines (https://score.depmap.sanger.ac.uk, accessed on April 2019). Remarkably, *FOSL1* is unique among *FOS*-family members, which (*FOS* and *FOSB*) are dispensable or (*FOSL2*) essential in only 1/323 lines [65], thus supporting the choice of Fra-1—among FOS proteins—as a target for therapeutic intervention.

5. Fra-1 in Drug Resistance and Drug Addiction Mechanisms

Together with the unique ability to seed new tumors, CSCs/TICs (Tumor-Initiating Cells) are refractory to anticancer treatments (drug- and radiation-resistant) and so responsible for clinical relapses [66]. The relationship between the EMT-associated transcriptional reprogramming and the gain of stem-like features, including drug resistance [67], is well-established. Therefore, therapeutic targeting of EMT-TFs via Fra-1 inhibition can not only contribute to the eradication of chemo-resistant CSC subpopulations [68], but also antagonize the radiation-resistant CSCs fraction.

BET (Bromodomain and Extra-Terminal domain) inhibitors are currently investigated in several clinical trials addressing hematological malignancies and solid tumors, including breast cancer. The promising therapeutic perspectives of BET inhibitors are hampered by multiple drug-resistance mechanisms, characterized in various preclinical models [69]. The role of Fra-1-containing dimers is suggested by a recent study based on multi-omics profiling and CRISPR functional screening, aimed at identifying the synthetic lethal and resistance interactions with the BET bromodomain inhibitor JQ1 in TNBC. In these cells, Fra-1 regulates its target genes mainly interacting with remote enhancers, which exhibit epigenomic and transcriptional profiles specifically associated with breast cancer subtypes [51][70]. Proteomic analyses by RIME (Rapid Immunoprecipitation Mass spectrometry of Endogenous proteins) show that Fra-1 participates in the BRD4-associated chromatin complexes. In addition, the synthetic-lethal interactions highlight the roles of the *Hippo* and AXL pathways in the resistance to the BRD4 inhibitor [71]. Significantly, Fra-1 cooperates with both the *Hippo* pathway, by interacting with YAP/TAZ/TEAD target promoters [72][73][74]), and the Gas6/AXL pathway, by transcriptionally inducing *AXL* [28][75]). Altogether, these data suggest that Fra-1 therapeutic inhibition might antagonize the acquired resistance to BET inhibitors.

Fra-1 accumulation in melanoma results from the mutationally activated RAS-BRAF-MEK-ERK pathway. Fra-1 triggers a switch in the expression of EMT inducers, involving the ZEB2 and SNAI2 downregulation, associated with the upregulation of ZEB1 and TWIST1, which drive the cancer cell reprogramming leading to melanocyte dedifferentiation and gain of mesenchymal features [76]. As in mammary and breast cancer cells [27][29], the *ZEB1* promoter is regulated by Fra-1, and ZEB1 is a Fra-1 effector in melanoma cells [76]. High levels of ZEB1, correlating with Fra-1 expression and melanoma stemness markers ($MITF^{lo}/p75^{hi}$ in CSCs vs $MITF^{hi}/p75^{lo}$ in non-CSCs) are implicated in intrinsic resistance to BRAF and MEK inhibitors. In addition, ZEB1 is overexpressed in melanoma cells with acquired drug resistance and in biopsies from patients relapsing while under treatment [77]. Therefore, Fra-1 inhibition might counteract the intrinsic or acquired melanoma resistance to BRAF and/or MEK inhibitors, by suppressing the ZEB1-regulated EMT-like transcriptional programs.

Along with key EMT regulators (*ZEB1* and *AXL*), Fra-1-containing dimers control the transcription of several miRNAs involved in therapeutic resistance. In ovarian cancer, the above-mentioned Fra-1-miR-134 autoregulatory loop causes decreased chemosensitivity to adriamycin and etoposide, because of the miR-134 effect on phosphorylation of the H2AX variant histone, which critically contributes to NHEJ-mediated DNA repair [45].

Although the above-described drug-resistance mechanisms point to Fra-1 inhibition as a tool for restoring the responsiveness to treatments, in specific conditions Fra-1 inhibition might be counterproductive.

In various neoplastic contexts, acquired resistance to targeted therapeutics depends on the compensating overexpression of some upstream component(s) of the RTK-RAS-BRAF-MEK-ERK signaling pathway. Drug removal results in in vitro growth arrest and in vivo tumor regression, due to the toxic effect of the rebound hyperactivity of the MEK-ERK pathway [78][79]. In melanoma cells exhibiting acquired vemurafenib resistance due to increased $BRAF^{V600E}$ expression, drug removal causes proliferative arrest, which indicates that drug-resistant cells have become addicted to vemurafenib [79]. Accordingly, melanoma patients with acquired resistance exhibit partial therapeutic responses when re-challenged with the same drug after interrupting the treatment [80].

The JunB/Fra-1 heterodimer contributes to the cell death caused by the overdose of MAPK signaling. Following drug removal from dabrafenib- and trametinib-resistant melanoma cells or EGFRi-resistant lung cancer cells, the Mek1/Erk2 rebound activity drives the JunB and Fra-1 accumulation, which triggers proliferative arrest and/or cell death [81]. In several MAPKi-resistant melanoma cell lines harboring different *BRAF* or *NRAS* mutations, the ERK hyperphosphorylation induced by drug withdrawal stimulates the p38-Fra-1-CDKN1A signaling axis, which results in p21 accumulation and proliferative arrest [82]. Moreover, conditioned media from drug-depleted vemurafenib-resistant cells inhibit the growth of untreated cells, thus suggesting the role of some growth-inhibitory secreted factor(s) regulated by Fra-1/JunB [83].

Therefore, in drug-addicted cells subjected to drug withdrawal, Fra-1 inhibition might favor rather than inhibit cancer cell survival. Namely, the clinical benefits resulting from intermittent treatments with RTK, BRAF, or MEK inhibitors could be lost, if *FOSL1* expression is suppressed in coincidence with the proliferative arrest triggered by drug removal.

In addition to conventional and targeted therapies, CSCs are also refractory to immunotherapy, because of the upregulation of immune checkpoint inhibitors such as PD-L1, associated with the presence of M2-polarized macrophages in tumor stroma [84]. Interestingly, Fra-1-containing dimers are involved in both mechanisms. In *KRAS*-transformed human bronchial epithelial cells, Fra-1 contributes to the escape from immune surveillance by mediating the MEK-ERK-

dependent induction of PD-L1 [85], while in TAMs, Fra-1 supports the polarization toward the M2 immunosuppressive phenotype [18][26].

6. Fra-1 in Drug Resistance and DNA Repair Mechanisms

In addition, other Fra-1-regulated mechanisms are implicated in resistance to targeted therapeutics. Based on the synthetic lethality between the loss of PARP activity and BER (Base Excision Repair) defects, PARP inhibitors, such as olaparib, allow the successful treatment of BRCA1/2 mutated cancers, although ineffective in BRCA-wild-type tumors, representing most (80–85%) of TNBCs. Remarkably, PARP1 has been identified among 118 chromatin-bound Fra-1 partners, by proteomic screening in TNBC cells [86]. The interaction between PARP1 and Fra-1 results in reciprocal inhibition. Consequently, while the olaparib-mediated PARP1 inhibition induces Fra-1 expression and activity, Fra-1 (and c-Jun) knockdown sensitizes the TNBC cells to the proapoptotic activity of the PARP inhibitor [87], thus suggesting that Fra-1 therapeutic inhibition could sensitize the BRCA-wild-type TNBCs to treatments with PARP inhibitors.

Researchers will examine several innovative strategies for targeting *FOSL1*/Fra-1 at multiple levels, including the Fra-1/AP-1 DNA-binding activity, *FOSL1* DNA sequence, and mRNA expression, Fra-1 stability, and transactivation mechanisms, along with the recent application of Fra-1-based suicide gene therapies.

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