Over-expressionofEFNA2inLung Adenocarcinoma:EFNA2geneexpression correlateswithshortenedsurvival

ABSTRACT

Background:Theincidenceoflung adenocarcinoma(LUAD)isincreasingworldwidewithdifferent prognosis.Ephrin-A2（EFNA2）,amemberoftheEph/ephrinfamily,isassociatedwithtumor progression.However,thecorrelationsof EFNA2withprognosisinLUADremainunclear.The purposeofthisarticleisto analyzetheimpactofEFNA2ontheprognosisofLUADpatientsthrough TCGA,Oncomine,andGEPIAdatabases,andtoexploreitspossiblemechanisms.

Methods:ThisarticlefoundasignificantcorrelationbetweenEFNA2andshortenedsurvivalin LUADpatientsthroughTCGA,Oncomine,andGEPIAdatabaseanalysis.Therefore,wefurther investedtherelationshipbetweentheexpressionandprognosticvalueoftheEFNA2 geneinLUAD patients.Sequentialdatafiltering(survivalanalysis,independentprognosticanalysis,andclinical correlationanalysis)wasperformed.EFNA2expressionwasanalyzedbytheOncominedatabaseand TumorImmuneEstimationResource(TIMER).WeevaluatedtheinfluenceofEFNA2onclinical

prognosisusingKaplan-Meierplotter,theprognoScandatabaseandGeneExpressionProfiling InteractiveAnalysis(GEPIA).ThecorrelationbetweenEFNA2andcancerimmune infiltrateswas investigatedbyTIMER.Inaddition,correlationsbetweenEFNA2expressionandgenemarkersetsof immuneinfiltrateswereanalyzedbyTIMERandGEPIA.Inaddition,geneenrichmentanalysiswas performed byMetascape.Finally,aco-expressionanalysiswasperformed bytheOncominedatabase.

Results: AcohortofLUADpatientsshowedthathighEFNA2expressionwasassociatedwithpoorer overallsurvival(OS),disease-freesurvival(DFS)byTCGA,andEFNA2wassignificantlyassociated withstageinLUAD.Inaddition, EFNA2expressionwaspositivelycorrelatedwithinfiltratinglevels ofBcellsandCD8+Tcells.Moreover,thedifferentialexpressionofEFNA2wassignificantlyhigherin lungadenocarcinomacomparedwiththatinnormalcontrols.Specifically,EFNA2waspositively associatedwithADAMTSL5,REEP6,PCSK4,C19orf25,andANAPC2.

Conclusions: OurdataindicatethatEFNA2isapotentialdiagnosticandprognosticbiomarkeranda promisingmoleculartherapeutictargettoattenuateLUADprogression.

Keywords:EFNA2,LUAD,prognosis,TCGA,ephrinA2

INTRODUCTION

"Lungcanceristheleadingcauseofcancer-relateddeathswithanincreasingincidenceoflung adenocarcinoma(LUAD)subtypeworldwide"[1].Prognosismayvaryinpatientswiththesamestagetumor becausecancerischaracterizedbygenetic,epigenetic,andphenotypic changesthat resultinatremendous variabilityinclinicalbehavior.Therefore,thedevelopmentofadditionalmolecularmarkersforsurvival predictionofLUADisrequired.Lungcancerisamalignanttumorcausedbyabnormalgrowthofbronchial cells,andprimarylungcancerispronetometastasis.Themostcommonpathologicaltypesincludesquamous

cellcarcinoma,adenocarcinoma,smallcelllungcancer,andsoon.Amongthem,LUADaccountsfor40%-55% ofthetotalnumberoflungcancers,mostofwhichoriginatefromthebronchialmucosalepithelium,and morethan3/4ofthepatients'lesionsoccurintheperiphery.Thediseaseprogressesslowly,andtheinitial symptomsaregenerallynotobvious,butitiseasytometastasize.

In recentyears,molecularprogresshas changedthetreatmentofLUAD, andgenetictesting hasbecome astandarddiagnosisandprognosticindicatorandcouldddeterminethetreatmenttarget.Theprogressof bioinformaticsandhigh-throughputsequencingwaswhetherwecouldidentifymanytumorbiomarkers, whichcouldhelpimprovetheaccuracyofpredictingtheprognosisofLUADandfindincreasinglyeffective treatments.EFNA2wasamemberoftheEphrinsfamily.Ephrins,ligandsfortheEphreceptors,its physiologicalrolenotonlyinvolvedcell-to-cellcommunication,celladhesion,cellmigration,andinvasion, butalsoinvolvedtheregulationofbloodvesseldevelopmentandangiogenesis.Theywerepromiscuousina verycomplexwebofrelationships(FIGURE1).Atpresent,thetargetedtherapyofLUADhasmade outstandingprogress,buttherewerestillabout10%ofpatientswithnegativegenetictesting,soweneeded moregenetictestingsitestoimprovetheprognosis.Andbettertargeteddrugscanbefoundbasedonthis gene.Currently,therearefewarticlesstudyingtheimpactofEFNA2onlungcancer.Onlyonearticlereports thatEFNA2canpredicttheprognosisofearlylungadenocarcinomainAsiansandCaucasians[2],andonlyone articlereportsthatthepreparedEFNA2targeteddrugs can increasethetherapeuticeffectof lung cancer[3]. Atpresent,researchontheimpactofEFNA2onlungadenocarcinomaisnotsufficient.Inthisarticle,we analyzedEFNA2andlungadenocarcinomathroughmultipledatabasesandanalysistoolsandfoundthat EFNA2isagoodbiomarkerforpredictingtheprognosisoflungadenocarcinomapatientsandisagoodtarget fortargetedtherapyoflungadenocarcinoma.

MATERIALSANDMETHODS

OncomineDatabaseAnalysis

WeanalyzedtheEFNA2 mRNAlevelsindifferenttumorsandnormaltissuesofmultiplecancertypesusing theOncominedatabase.(https://www.oncomine.org/resource/login.html).Thethresholdwasdetermined accordingtothefollowingvalues:P-valueof0.001,foldchangeof1.5,andgenerankingofall.

DataDownloadandPreprocessing

GeneexpressiondataandcorrespondingclinicaldatafromLUADpatientsweredownloadedfromTCGA (http://www.cgga.org.cn/).Thisdatasetthatcontained 594samples(DataSetID:mRNAseq_594,Data Type:RNAsequencing)weredownloaded.ThegeneexpressiondatafromLUAD sampleswerecorrectedin batchesandintegratedbyloadingthemintothelimma(14)andsva(15)packagesinRsoftware(Rversion 3.6.1:https://www.rproject.org/).

SurvivalAnalysisFiltering

SurvivalandsurvminerpackageswereloadedinRsoftware,andKaplan–Meier(K-M)(16)andunivariate Coxanalyseswereused tofiltergeneexpressiondataandsurvivaldataat asignificancelevelofP<0.05.

IndependentPrognosticAnalysisFiltering

Thegeneexpressiondataobtainedfromthesurvivalanalysisandintegratedclinicalinformationwere analyzedusingmultivariate CoxanalysiswithRsoftware,atasignificancelevelofP<0.05.

AnalysisoftheCorrelationBetweenEFNA2ExpressionandClinical Characteristics

92 ThecorrelationbetweenEFNA2expressionandvariousclinicalcharacteristicswasplottedusing

93 UALCAN(http://ualcan.path.uab.edu/analysis.html).

94

95 ## GepiaDatabaseAnalysis

96 “TheexaminationofEFNA2expressioninhomogeneoussubsetsofLUADwasperformedinGEPIA.GEPIA,an

97 interactivewebservercontainingRNAsequencingdatabasedon9,736tumorsamplesand8,587normal

98 samplesfromtheTCGAandGTExdatabases,providescustomizablefunctionssuchastumor/normal

99 differentialexpressionanalysis,patientsurvivalanalysis,andcorrelationanalysis”. [59]

100100

101 ## GeneEnrichmentAnalysis

102 Inthisstudy,Metscapewasusedtogenerateanorderedlistofallgenesassociatedwiththeexpressionof

103 EFNA2.Then,Metscapewasusedtoidentifysurvivaldifferencesbetweenthehighandlow EFNA2groups.

104104

105 ## AnalysisofImmuneInfiltration

106 “TumorImmuneEstimationResource(TIMER,https://cistrome.shinyapps.io/timer/)wasusedto

107 comprehensivelystudy themolecularcharacteristicsoftumor-immuneinteractions”(18).“Theabundances

108 ofsiximmuneinfiltrates,includingB cells,CD4+Tcells,CD8+Tcells,macrophages,neutrophils,and

109 dendriticcellswereevaluated.Weanalyzedtherelationshipbetweentheexpressionlevelof EFNA2and

110 thelevelofimmuneinfiltrationinLUADusingtheTIMER“gene”module.TheKaplan-Meiermethodwas

111 usedtoplottheeffectofEFNA2expressionandimmunecellinfiltrationontheprognosisofpatientswith

112 LUAD,andclinicalfactorswereincludedtoconstructamultivariateCox proportionalriskmodel.Finally,

113 therelationshipbetweencopynumbervariations(CNVs)of EFNA2indifferentsomaticcellsandthelevel

ofinfiltrationinLUAD wasanalyzedusingthe“SCNA”module”.[59]

Co-expressionAnalysis

Oncomine(https://www.oncomine.org)wasusedtoscreengenesthatwereco-expressedwithEFNA2.In addition,thepheatmap(https://github.com/taiyun/corrplot)packagewasusedtoplotthefirst20genes positivelyandnegativelyassociatedwithEFNA2.TheCorrplot(https://github.com/taiyun/corrplot)and CirclizepackagesinRwereusedtogenerateacircularplotofthetopfivegenespositivelyandnegatively associatedwithEFNA2.

RESULTS

TheExpressionLevelsofEFNA2inDifferentTypesofHumanCancers

TheexpressionleveloftheEFNA2geneinvarioustypesofcancerswasidentifiedintheOncomine database.ThisanalysisrevealedthattheEFNA2expressionwashigherinlungcancercomparedtonormal tissues(**Figure2A**).TofurtherevaluateEFNA2expressioninhumancancers,weexaminedEFNA2 expressionusingtheRNA-seqdataofmultiplemalignanciesinTCGAdeterminedbyTIMER.Thedifferential expressionbetweenthetumorandadjacentnormaltissuesforEFNA2acrossallTCGA tumorsisshownin **Figure2B**.EFNA2expressionwassignificantlylowerinLUAD(lungadenocarcinoma) comparedwith adjacentnormaltissues.

Kaplan-MeiersurvivalanalysisoftheTCGAdatasetshowedthatlowEFNA2expressionwasassociatedwith betterprognosisinpatientswithlung adenocarcinoma(**Figure3A**).UnivariateCoxanalysisshowedthat

EFNA2(HR= 1.065;95%CI = 1.022-1.110;P<0.05),T stage,Nstage,M stageandTNMstage werehigh-riskfactors,andpathologywerelowriskfactors(**Figure3B**).MultivariateCoxanalysisshowedthatEFNA2 (HR=1.053;95%CI= 1.007–1.102;P<0.05)wasindependentlyassociatedwithoverallsurvival,which suggestedthatEFNA2couldbeanindependentprognosticindicatorforlungadenocarcinoma.Inaddition, pathologyandTNMstagemayalsobeindependentprognosticfactors(**Figure3C**).

AnalysisoftheRelationshipBetweenEFNA2ExpressionandClinical Characteristics

Analysisof573samplesfromtheTCGAdatabaseshowedthatthedifferentialexpressionofEFNA2was significantlyhigherinlungadenocarcinomacomparedwiththatinnormalcontrol byusingUALCAN[4] (**Figure4A,**P<0.001).Wealsocanseethesignificantdifferentbetweennormaltissuesand41-88years, differentstages,gender,N0-N3nodalmetastasis,differentraces,NOS,mixedandmucinoushistological subtypes,differentsmokinghabits,differentTP53mutations (**Figure4B-I**).Inaddition,analysisof573 samplesfromtheTCGAdatabaseshowedthatthedifferentialexpressionofEFNA2wassignificantly associatedwithpatientsraceandhistologicalsubtypes(**Figure4F**).TheEFNA2expressionofCaucasianwas significantdifferenttoAfrican andAmerican (P<0.001)orAsian(P<0.05).AndtheEFNA2expressionof mixedsubtypewassignificantdifferenttolungbronchioloalveolarcarcinoma,non-mucinoussubtype(P< 0.05) orlungsolid patternpredominantadenocarcinomasubtype(P <0.05).TheEFNA2 expression of lung adenocarcinomanot otherwisespecified(NOS)subtypewassignificantdifferenttothelungsolidpattern predominant adenocarcinomasubtype(P<0.05).

RelationshipBetweenEFNA2ExpressionandPrognosisofLUADPatients

GEPIAisapublicdatabaseestablishedforexpressionprofilinganalysisofcancerandnormalgenes.[5]

PrognosticanalysisrevealedthathighexpressionofEFNA2wouldleadtoashortoverall survivalinpatients withLUADbasedonGEPIAdatabase(**Figure5A**,P<0.05).Inaddition,EFNA2wassignificantlyassociated withstagein LUADanalyzedbyGEPIA database(**Figure5B**,P<0.05).

AnalysisoftheCorrelationBetweenEFNA2ExpressionandClinical CharacteristicsinTCGADatabase

AnalysisusingTIMERshowedthatEFNA2wasnegativelyassociatedwith BcellsandCD8+Tcells(**Figure6)**. Univariatecoxsurvivalanalysisshowedthat EFNA2,Bcell,anddendriticcellswereassociatedwiththe survivalofpatientswith LUAD(**Figure7)**.Furthermore,onlyarmleveldecreasesin copynumbervariations (CNVs)ofEFNA2wereassociatedwiththeextentof immuneinfiltrationin LUAD immunecells(**Figure8)**. TheseresultsshowedthatEFNA2wasassociatedwithimmuneinfiltrationinLUADusingexternaldata analysis.ThesefindingsindicatedthatEFNA2mightbeaprognosticbiomarkerof LUAD andmaybeatarget forimmunotherapy.

GeneEnrichmentAnalysisofEFNA2

"Geneenrichment analysisisacomputationalmethodusedto determinewhetheragroupofgenesis differentiallyexpressedintwobiologicalstates.Metascapegeneenrichmentanalysiswasusedtoidentify GO(GeneOntology)andKEGGsignalingpathwaysthatwerediffer entiallyexpressedinLUADbetweenthe lowandhighEFNA2expressiongroups.Theresultsshowedsignificantdifferencesinenrichmentusing Metascape" (http://metascape.org/).Themostsignificantly enrichedGOandsignalingpathwayswere selectedbased onpvalue.Asshownin**Figure9**Aregulatedexocytosis,bloodvesseldevelopment,cell

cyclephasetransition,geneontologyterms,extracellularmatrixorganization,cellcycle,mitotic, hemostasis,collagenformation,PIDintegrin1pathway,nervoussystemdevelopmentsignalingpathway wereenrichedintheEFNA2highexpressionphenotype.

Co-expressionAnalysisofEFNA2

Aheatmap(**Figure10A**)ofthetop20genespositivelyandnegativelyassociatedwith EFNA2wasplotted. Inaddition,acircularplot(**Figure10B**)ofthetopfivegenes positivelyandnegativelyassociatedwith EFNA2wasgenerated.TheresultsshowedthatEFNA2waspositivelyassociatedwithADAMTSL5,REEP6, PCSK4,C19orf25and ANAPC2,andwasnegativelyassociatedwithMSLNL,SLC35F2,RAB39B,BIRC3and KIAA1377.

Discussion

ThetreatmentmethodsofLUADincludedsurgery,radiotherapy,chemotherapy,targetedtherapy,and immunotherapy.Atpresent,surgery,radiotherapy,andchemotherapyhavebeenusedformanyyears,and thetherapeuticeffect has beenbasicallyapplied tothelimit.This requiresustoexplorenew treatment directions.Inadditiontoexternalenvironmentalfactors[6],themost importantcauseoflungcanceris geneticchanges[7,8].“Therefore,westudiedthe relatedgenes ofLUAD,hoping to findnewtreatment methodsorprognosticfactors.WeshowedthatEFNA2wasahigh-riskfactorandcouldbeanindependent prognosticindicatorinpatientswith LUADusingcomprehensiveunivariateandmultivariateCox analyses. Takenaltogether,theseresultsindicated thatEFNA2wasupregulatedinLUAD,and EFNA2hada prognosticvalueinLUAD,indicatingthatEFNA2hadimportantregulatoryfunctionsinLUAD.EFNA2isa memberoftheephrinfamily whosegeneshavebeenreportedto befrequentlyoverexpressedinawide varietyofcancertypesdirectlyregulatingcriticalstepsofcellularadhesion,tumorgrowth,chemo-

repulsion,invasion,metastasis, angiogenesis, axon guidance,tissueborderformation"[9,10][11,12][13-16][17-28].

TherelativestudiesshowedthattheexpressionlevelsofEFNA2werenegativeandcorrelatedwiththe prognosisof prostate cancer[29],CD133 highneuroblastoma[30,31]， breastcancer[15]， hepatocellular carcinoma[14],gastriccancer[32]andcolorectalcancer[33].Forexample,Fengetal.foundthat"EFNA2is significantlyupregulatedinboth cancerouscelllinesandclinicaltissuesamplesofhepatocellularcarcinoma (HCC)andcomparedwiththenormalones"[14].Chakraborty'sstudyfoundthatEFNA2Trp112Cysmutantmay beacontributingfactortothedevelopmentofnon-smallcelllungcancer [34].In addition,Fox etal. reportedthat"theexpressionofEFNA2issignificantlyhigherinCPTXcells(humanlocalprostatetumor) comparedtoNPTXcells(normalhumanprostate epithelium),suggestingthatEFNA2 maypromotethe transformationofthenormalprostateepithelialcellintoonewithamalignantphenotype"[16]. Zhaofound that"ectopicexpressionofEFNA2canpromotetheinvasionandmetastasisofprostatecancercells, promotebloodvesselproliferation,andsilenceEFNA2,whichcanreducetheinvasivenessandmetastasis ofprostatecancercells"[35].Inaddition,EFNA2participatesintheregulationofdiversecellularprocesses andgeneexpressionthroughchromatinremodeling,andtheexpressionofEFNA2atthetranscriptionlevel wouldleadtotheactivationofsignalingpathwaysrelatedtotumorprogression.Forexample,Liuetal. showedthat"blockingEFNA2expressioninhibitsthemetastasisabilityofhumanlivercancercellline HepG2"[36].Kuodiscoveredthat"EFNA2canpredicttheprognosisofearlylungadenocarcinoma".[2]"Moreover, ephrinsfamilyviaEphrinreceptor(Eph)–ephrininteractionsregulatecriticalstepsof angiogenesis,blood vesselformationmalignant transformation, tumormetastasis,tumordifferentiation,andoutcome"[17-19,27, 37-40]."Forexample,upregulationofEphA2hasbeenobservedinmanymalignanttumorsandis associated withacceleratedcellproliferation,stimulatingangiogenesis,andpromotedcellmigrationandinvasion, increasingcancercellsurvival"[17-27].PsilopatisreviewedEphrin/Ephfamilytargetedtreatmentof

gynecologicaltumors,breastcancerandlungcancer[41-43],PapadakosreviewedEphrin/Eph familytargetedtreatmentofcoloncancer[44],HadjimichaelreviewedEphrin/Ephfamilytargeted treatmentofboneandchondrosarcoma[45],andfoundthatEphrin/Ephfamilytargeted treatmentcannotonlypromotetheregressionoftumors,butalsoincreasetheeffectof radiotherapy,chemotherapyandtargetedtreatment[34],whichisanewhopefortumor treatment,Morethantendrugshavebeendiscoveredinthetreatmentoflungcancer.Huanghas preparedadrugtargetingEFNA2,whichhasasignificantkillingeffectonlungcancercellsand hasenteredphaseIclinicalresearch[3]. “Inaddition,researchandclinicaltrialshaveconfirmedthe proteolyticsheddingofmembrane-boundEphrin-As,whichreleasessolublefragmentsatthecellular level”[46-48].“Forexample, membranebound EFNA2hasbeenidentifiedasthesubstrateof ADAM10and releasedsolubleEFNA2fragmentsintothe cellmedium”[46,47].“Thesedata suggested thatsecretedEFNA2 maybeusefulserummarkersforthediagnosisandprognosisofmanytumors”[49].

“Besides,geneenrichmentanalysis wasperformedtoobtainfurtherinformationabouttheroleofEFNA2in tumorprogression.TheresultsofTIMERshowedthat aregulatedexocytosis,bloodvesseldevelopment, cellcyclephasetransition,Geneontologyterms,extracellularmatrixorganization,cellcycle,mitotic, hemostasis,collagenformation,PIDintegrin1pathway,nervoussystemdevelopmentsignalingpathways wereenrichedintheEFNA2highexpressionphenotype.Theextracellularmatrixregulatestissue developmentandhomeostasis,anditsdysregulationcontributestoneoplasticprogression.The extracellularmatrixservesnotonlyasthescaffolduponwhichtissuesareorganizedbutprovidescritical biochemicalandbiomechanicalcuesthatdirectcellgrowth,survival,migration,anddifferentiationand modulate vascular developmentandimmune function”.[50,51]

“Thegeneontologyterms ofEFNA2weregenerallyenrichedinBcellrelatedmediatedimmunity,humoral

246 immuneresponse,andinnateimmuneresponse.Blymphocytewasrecognizedtoparticipateinregulating

247 theimmuneresponsetomurineandhumantumors”[52].“RegulatoryBcellplayan immunosuppressiverole

248 incarcinogenesisandbecomeatherapeutictargetinsolidtumors[53].RecentstudiesindicatedthattheB

249 lymphocytesexistsinallstagesofcancerandplaysimportantrolesinshapingtumordevelopment inlung

250 cancerandthusinfluencestheprognosisoflung cancerpatients”[54,55].

251 “Finally,co-expressionanalysisshowedthatEFNA2 waspositivelyassociatedwith ADAMTSL5,REEP6,

252 PCSK4,C19orf25,and ANAPC2,andwasnegativelyassociatedwithMSLNL,SLC35F2,RAB39B,BIRC3and

253 KIAA1377.Aspreviouslyreported, ADAMTSL5wasanepigeneticallyactivatedgeneunderlying

254 tumorigenesisanddrugresistanceinhepatocellularcarcinomaandpointedtoaroleforADAMTSL5in

255 maintainingthefunctionofkeyoncogenicsignallingpathways,suggestingthat itmayactasamaster

256 regulatoroftumorigenicityanddrugresistance”.[56].Moreover,proliferationandmetastasisof lungcancer

257 cellslackingREEP5andREEP6weremarkedlydecreasedcomparedtothecontrolgroup,andthey couldbe

258 novelregulatorsofG-protein-coupledreceptorsignaling[57].Thesereportssuggestedthat ADAMTSL5and

259 REEP6wereassociatedwiththeregulation ofproliferationandmetastasis ofcancer.Ourstudyshowed

260 EFNA2tobeassociatedwithADAMTSL5andREEP6,whichindicatedthatEFNA2mightbeassociatedwith

261 theregulationofproliferationandmetastasisofcancer.

262 Ephsandephrinswereregardedaspromisingcandidatesfordrugdevelopment.However,Ephandephrin

263 hadbeenconsideredasundruggabletargetmoleculesbecause theinteractionsbetweenEphandephrin,

264 showninFigure1,werenot specificandpromiscuous inaverycomplexwebofrelationships.Many

265 processesthatinvolvefastchangesincellularmotilityand/ormorphologydepend onephrin–Ephsignaling

266 pathway.Therefore,therearenodrugsagainsttheEph/ephrinfamilyformedicaluseincludedinthe

267 clinicalguidelinessofar.Dasatinibistheonlydrugformedicalusethat showsaninhibitoryeffectonEphA2

activity[58].However,dasatinibhasnot beenusedforanyanti-EphA2therapysofar.Recently,Richard Huangetal.discoveredEFNA2targetedimmunoliposomesincorporatingpH-sensitivetaxaneprodrugs weredevelopedforsustaineddeliveryofactivedrugstosolidtumors,andthisdrughadenteredaPhaseI clinicaltrial[3].TheseresultsindicatedthatEFNA2maybeausefulmoleculartherapeutictargettoattenuate LUADprogression.

WespeculatedthatEFNA2maybeusedasaprognosticindicatorforLUAD,and futurestudieswillbe neededtoexploretheprotein inamultidisciplinarywayinthefuture,hopingtofindamolecularpredictor withgreatclinicalvalue.

CONCLUSION

This study looked into the connection between EFNA2 and the prognosis for LUAD. Initially, sequential. The important gene EFNA2 was screened via data filtering. After then, EFNA2 was examined for a relationship withoutcome as well as clinical features. The findings indicate that elevated EFNA2 expression was linked tolower prognosis, and EFNA2 was a high-risk factor that could be utilized on its own to predict outcomes. Indicator for LUAD sufferers. Additionally, an examination of Metascape gene enrichment revealed that EFNA2potentially control LUAD's growth and metastasis, and EFNA2's overexpression in LUAD suggestsbleak prognosis. Cell-to-cell contacts within tumor cells and the tumor itself are mediated by EFNA2. Microenvironment, specifically the vascular and tumor stroma. Consequently, EFNA2 has been regarded asdesirable objectives for medication design, as targeting.

DATAAVAILABILITYSTATEMENT

Thedatathat support thefindingsofthisworkareobtainablefromthecorrespondingauthorbasedon reasonablerequest.

AUTHORCONTRIBUTIONS

ChunmeiLiu wrotethemanuscriptandperformedbioinformaticsanalysis.YanjiaoWu,HuandiZhouand XiaohuiGecontributedtomanuscriptdiscussion.XiaoyingXueand GuohuiWangdesignedthestudy, researchedtheliterature,andcontributedtothefiguresandtables.YanjiaoWu,HuandiZhou,andXiaojing

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Figurelegends

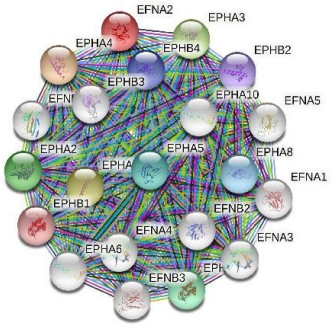

FIGURE1|EstablishmentofthePPInetwork.

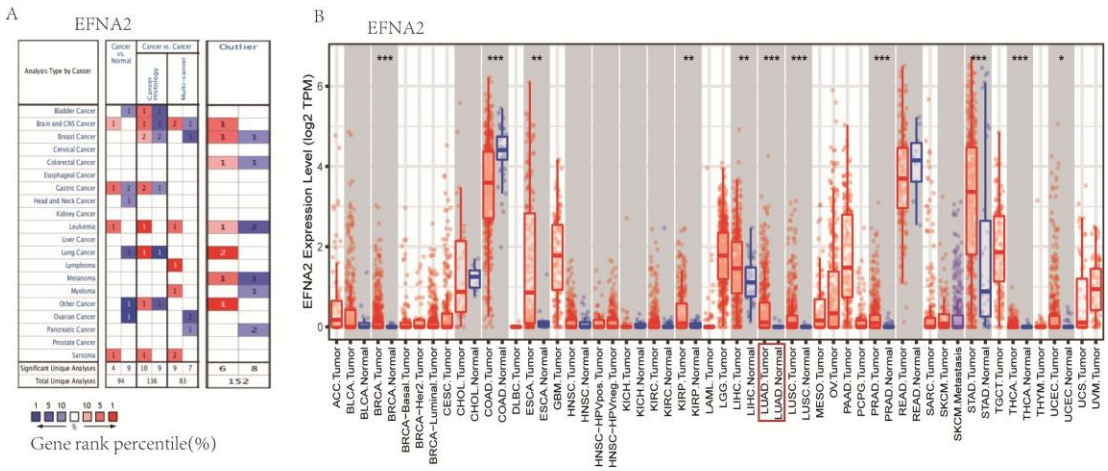

FIGURE2|EFNA2expressionlevelsindifferenttypesofhumancancers. (A)IncreasedordecreasedEFNA2indatasetsofdifferentcancers

468 comparedwithnormaltissuesanddifferentpathologyofcancerintheOncominedatabase. (B)HumanEFNA2expressionlevelsindifferenttumor

469 typesfromTCGAdatabaseweredeterminedbyTIMER.(*P<0.05, **P<0.01,***P<0.001).

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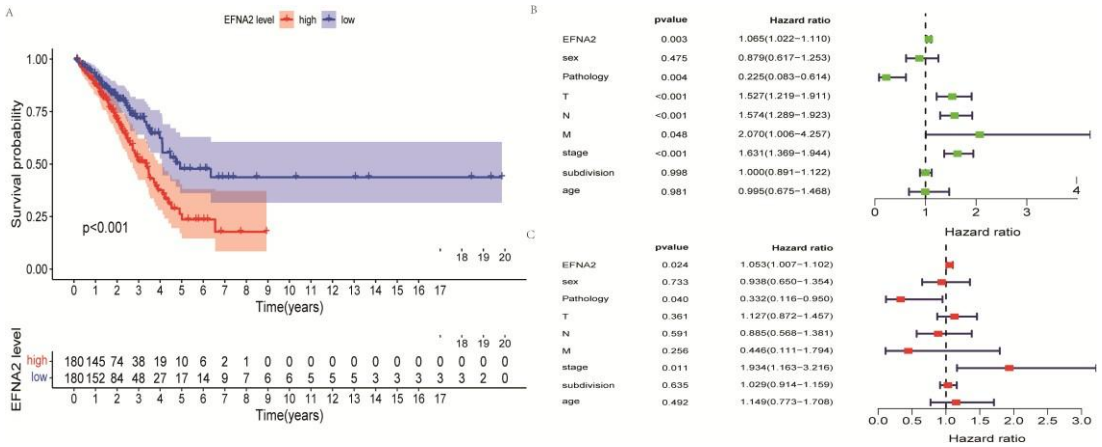

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472 FIGURE3|Bioinformatics analysisof EFNA2usingtheTCGAdatabase(LUAD, n=367). (A)Survivalanalysisofpatientswithlung

473 adenocarcinomainthehighEFNA2andlowEFNA2groups. Redindicates highexpressionandblueindicates lowexpression.P<0.001. (B)

474 UnivariateanalysisofEFNA2.(C)MultivariateanalysisofEFNA2.

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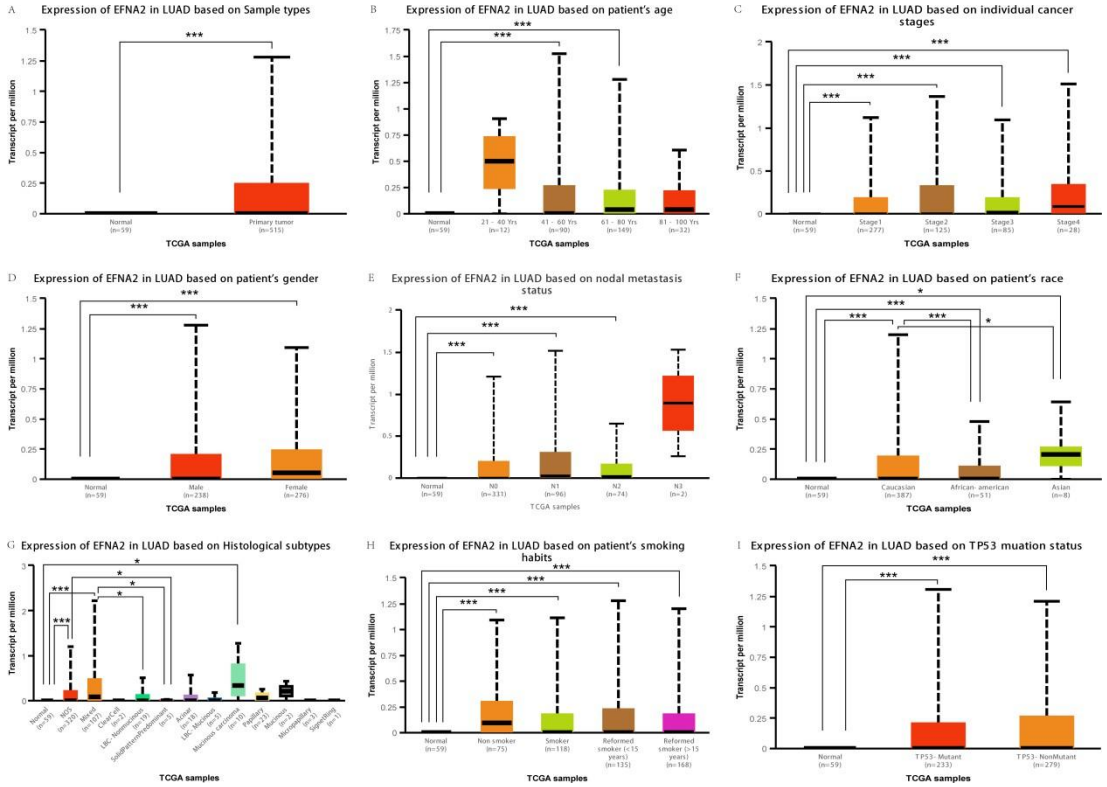


FIGURE4|RelationshipBetween EFNA2Expressionbasedondifferentfactors oflungadenocarcinomaPatients.(A)EFNA2Expressionbased onTCGAsampletypes. (B)EFNA2Expressionbasedonpatient'sage.(C)EFNA2 Expressionbasedoncancerstage.(D)EFNA2Expression basedonpatient'sgender.(E)EFNA2Expressionbasedonnodalmetastasis. (F)EFNA2Expressionbasedonpatient'srace.(G)EFNA2 Expressionbasedonhistologicalsubtypes.(H)EFNA2Expressionbasedonpatient'ssmoking.(I)EFNA2ExpressionbasedonTP53mutation status.(*P<0.05,**P<0.01,***P<0.001).

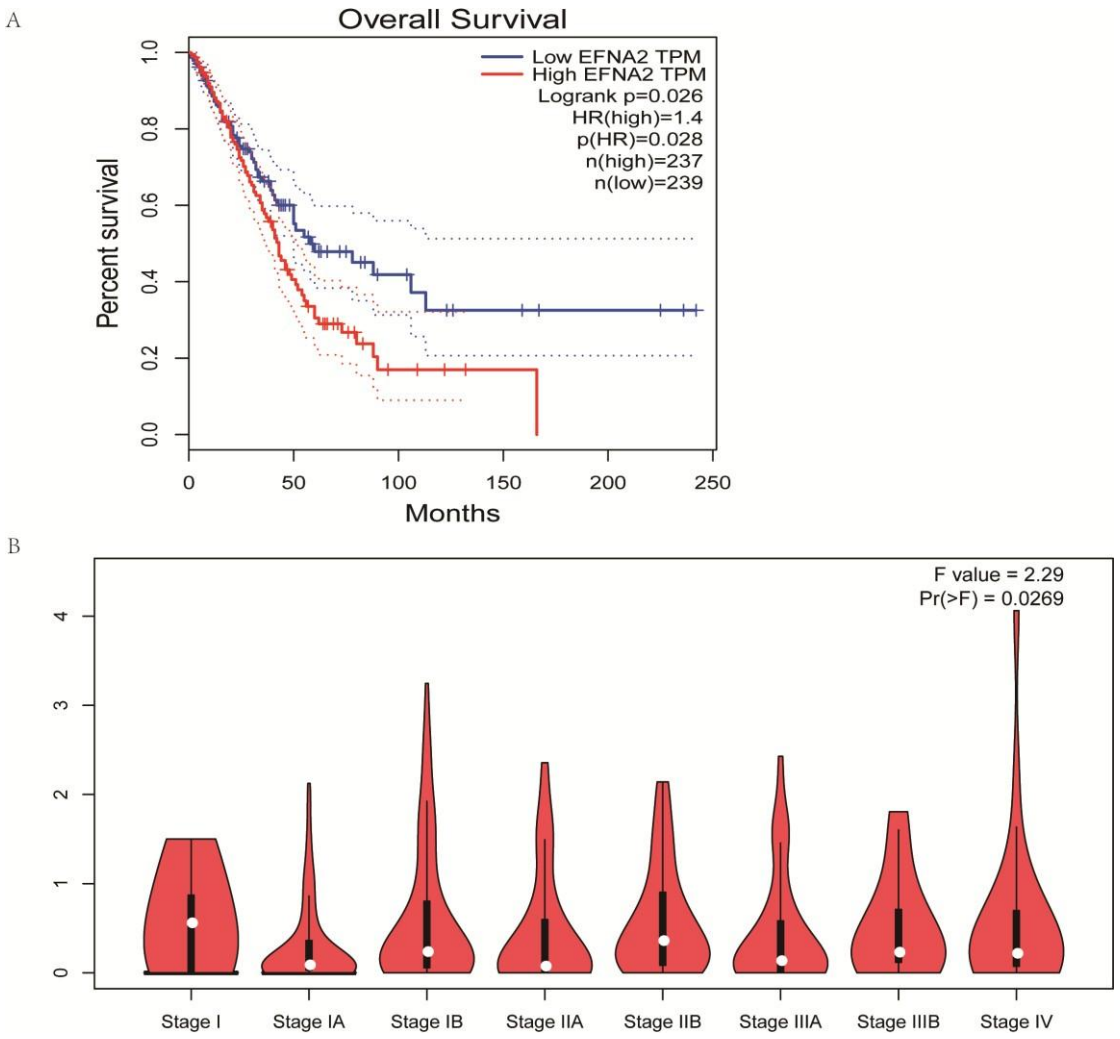


FIGURE5|RelationshipbetweenEFNA2expressionandprognosisofLUADpatientsbasedonGEPIAdatabase.(A)Therelationshipbetween EFNA2expressionlevelsandoverallsurvivalinLUADwasanalyzedbyGEPIAdatabase. P<0.05.(B)EFNA2wassignificantlyassociatedwith stageinLUAD analyzedbyGEPIA database. P<0.05.

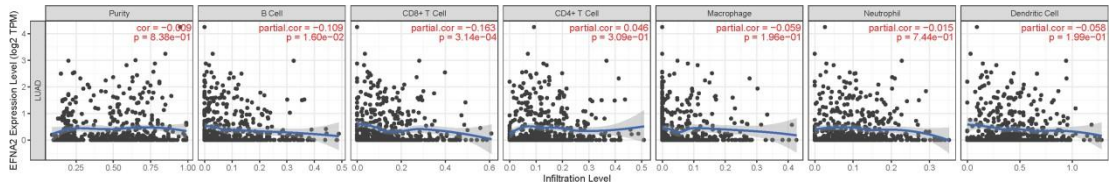
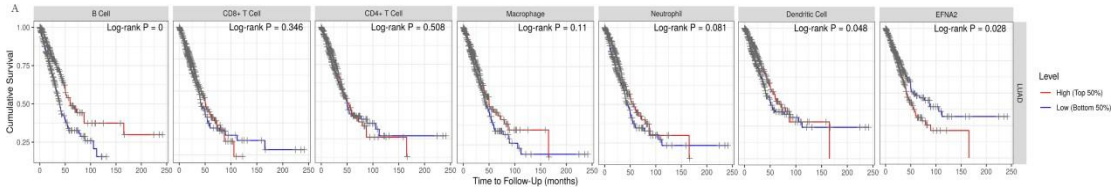


FIGURE6|Correlationbetweentheexpressionof EFNA2andimmuneinfiltrationof LUADcells.



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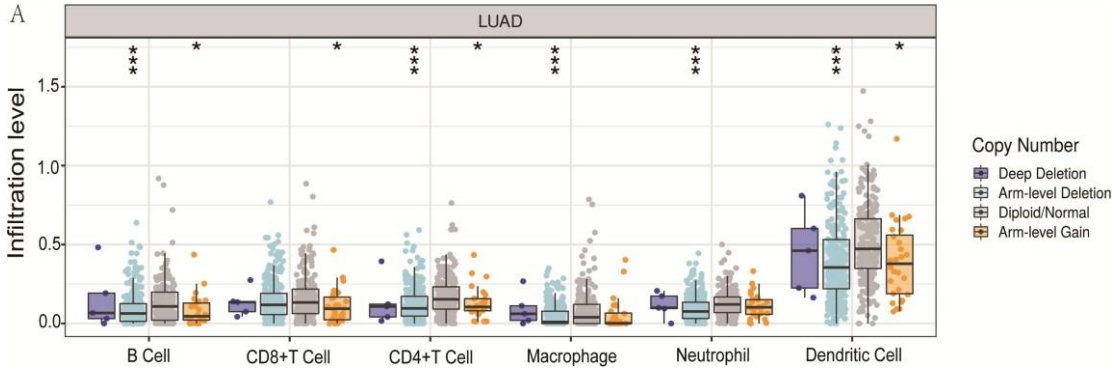
492 FIGURE7|Survivalcurveforimmunecellinfiltration.Kaplan–Meiersurvivalcurvesbasedontopandbottomsamplepartitionswith50%

493 immunepenetration. Redindicates ahighdegreeofinfiltrationandblueindicates alowdegreeofinfiltration.P<0.05wasconsideredsignificant

494 andP<0.0001was reported as0.

495



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497 FIGURE8|Relationshipbetweencopynumbervariationof EFNA2 andimmuneinfiltrationlevelin LUAD. *P<0.05;***P<0.001.

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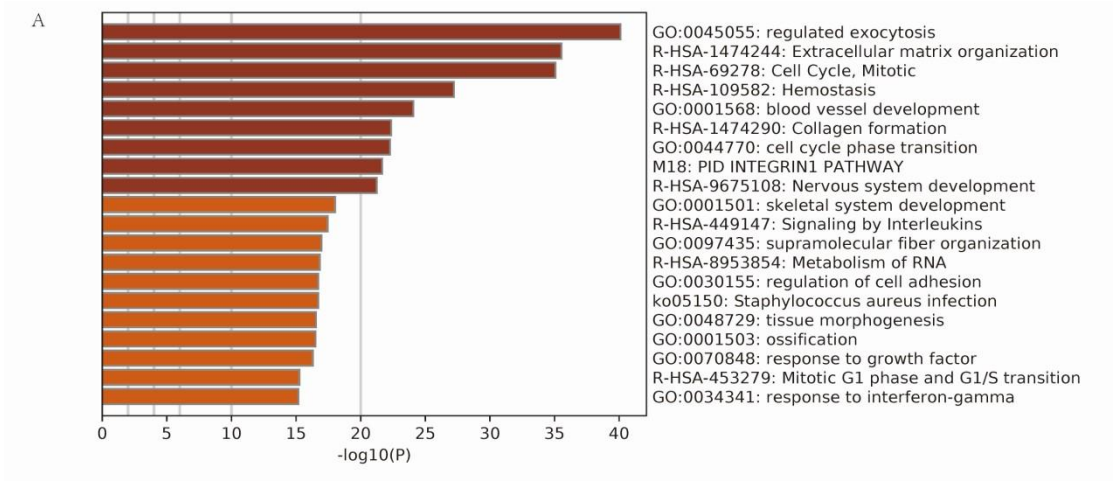

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500 FIGURE9|GeneSetEnrichmentAnalysisof EFNA2.Heatmapofenrichedtermsacrossinputgenelists, coloredbyp-values.

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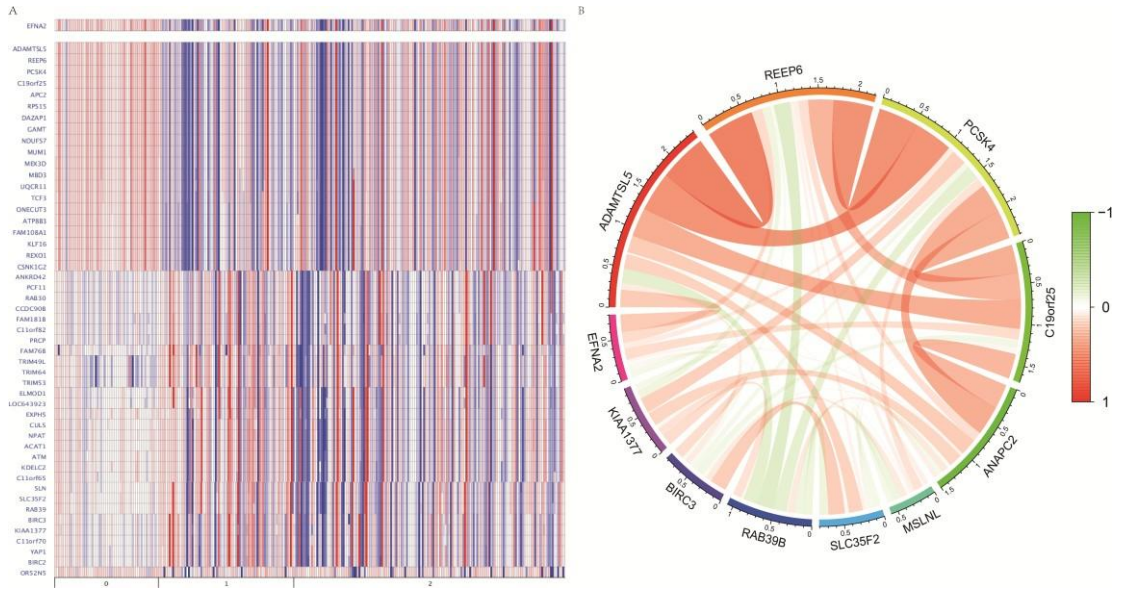

FIGURE10|Co-expressionanalysisofEFNA2usingtheTCGAdatabase.(A)Heatmapofthetop20genespositivelyandnegatively associated withEFNA2.(B)Circularplotofthetopfivegenespositivelyandnegativelyrelatedto theEFNA2gene.Greenrepresentsnegativeassociation, andredrepresentspositiveassociation.