The effects of heat shock to catenins and epidermal growth factor receptor in gastrointestinal cancers

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/ @¡ B / / ”K›n

§Q¥ ¸ › G ø † › M fi Ł ' ¤ ¤ … ¥ § J ⁄ ¸ ⁄ W ¥ ¥ ˝ ˝ ł ƒ ƒ]l¡A¤ ¤ ¯Ø˝[1,2,3]¡C catenin¡A E-cadherin ˝ †{˝˝§ ¯ ‹ ª ¤s¡A ϕ{… ¥ §„catenin˝ ˝ v.¯¤T^ ⁄ £ ª ¡A ‹O⁄ W ¥ ¥ ˝ ˝ ł ƒ ƒ]l– ¤ ƒ¤ ´ ' E-cadherin˝ ˝ v.¯¤T §e†{ƒ ‡ N,˝q˝ fi t §¡C

/G¡B›^/ K›n

Using three gastric cancer cell lines (SC-M1, NU-GC-3, AGS), the E-cadherin and catenin expression was detected under the treatments of heat shock and epidermal growth factore receptor. The catenin expressions were not significantly affected by heat shock treatment, but the concentration of soluble E-cadherin was significantly changed after the treatment of epidermal growth factor receptor.

/T¡B‰¥

Catenin ‹O ƒb † ›M ⁄ §P E-cadherin ‹ ‡s– ˝˝⁄ @ ˝ ƒ¥ ‰Ł [1,2,3]¡C E-cadherin … s ¤ƒƒb' ⁄W›F … h ¤•‰ ˝ † ›M⁄¡C E-cadherin »P† › M˝§ ¸ ˝ A ⁄⁄ƒ‡^ «Y¡A¤ ˝ †{»P ø ƒ¤ø„ ´ †fi§fî§¡A§ »P øfl g fl–W˝„w «Æƒ‡^ ¡C E-cadherin ˝˝¥ ƒl »P catenin ˝˝¥ ƒl fi §§‹ ^ ¡C•U¤ • Ufh˝˝„ Œ ¯^ ¯ª¥ ¡A⁄ W ¥ ¥ ˝ ˝ ł ƒ⁄l– ¤ ¯Ø ¡]EGFR¡^˝˝` ø˜⁄˘¡A‡ ‚L»P beta- ' ¸gamma-catenin ˝˝¥ ‹§¥¸¡A»P⁄ _ E-cadherin/catenin ‰ ƒX¯ Ø˝⁄£‹ ¡ ⁄˘ƒ‡^ ¡C

§ › ¥ ˝‹e„ Œ ⁄ w œ˝› G ø† ›M• |˝ †{E-cadherin¡C§ › ¥ «e⁄w·œ ˝ › G ϕ ˝›M†{ E-cadherin• |¤ ¤ … ¥ § J˝ƒ..v§¯T ¡A‡ ˝æ§ ϕ{›G ø † ›M catenin˝˝˝ †{⁄ ‡£⁄£˝ ‹ ƒP¡C ¡˜⁄˝ ˝ • ¯ł]..k ¥ §J˝‡¥ ¸¡^ ⁄w‡Q¥ ¸¤ ˝ vł›G, ‚D øƒX¤ ¡ ⁄˝ ´ † ˝˝flƒ¤ ¡Cƒ„˝ ˝ v ł†¡˝A¥ifl ƒ‡ ‡¡⁄ › ƒ]‹O… ¥§•J|§ ¯ ›G, ‚D ø † ›M˝ ›–„ ' E-cadherin˝ †{ ‹ ˝˝ § ¯ ¡C§ › ‡]… ¥§J]–N§ ¯ ›G, z „D ø † ›MEGFR⁄ ¸catenin˝˝˝ †{ { « ¡Cƒb¥»× ª s⁄¡A§ › ¥ h»·œ›G, ‚D

ø † M⁄Æ"cadherin, catenin, EGFR, phosphorylated tyrosine residues, EGFR-tyrosine kinase ƒ b …¥ S J « e Æ⁄Ł'ƒP † { {« ¡C

¥ | ¡ B § tf⁄Ł"k

S › ƒP... ¸ S Q ¥ ¸ @ ›"⁄Ø¥» ›G ø † xMŁNU-GC-3 [4]¡A⁄ @ ⁄⁄ Œ "⁄ H ^ ›G ø † xMŁSC-M1 [5]¡A¥ H¸ ⁄ @ ‹ Œ"⁄ H ^ ›G ø † xMŁAGS [6] ¤ S @ ƒ‹¡ª©s⁄⁄NU-GC-3 ‹ O qƒ ƒ ⁄ ´ ¤ i¥X˘⁄£¤}"⁄´ †'° ›G, ¢ ø † ›¦M‹ ƒ h⁄S¸ i'1¡G 1 "⁄ RPMI-1640 »P Dulbecco's modified Eagle's medium (Gibco®)†V ƒ X † @"S¦0%" ⁄ › L ⁄ ƒ S¦M¦l « h ‹ O – q › G ø † ⁄»⁄"›G , ¢ø¡CSC-M1 i' RPMI-1640 (Gibco®, Grand Island, NY)¡CAGS «h ‹ O i'Ham's F-10¡C¥ H W"⁄ i ‹ S ƒ ł0%"⁄› L ⁄ ƒ ¡ŒMo ¤ ˙ † ›M†£ ‹ O ƒb 37¢ JA5%CO2-95% air¡C † ›M"⁄... ¥ S†ŒG†N i⁄⁄ "⁄† ›M¦A‡ ƒP i¥ ⁄ @ ¥[42.5 ± 0.1¢J [7]¤ ^ ø˜30 ⁄ ˜`¡A60 ⁄ ˜` ⁄¸120 ⁄ ˜`⁄ £¥¦A¥tƒ‡⁄@† £,¥g .[. ł Bz¥HS @ ‹ „•¦C¥† .[. ¥H«Æ"⁄† ›M ¥H 2mM ethylenediaminetetraacetic acid¡]EDTA¡ ^ »†ŒG Bz¤ ˘ ¤ ›Ø¡A¤ ¤^¥HTrypan Blue ‹ ¥ ¡A¤^ƒb¯ ª Ł^Ł ⁄ U ‛„¤ ƒ s ‹ †v¸› p˘¡C † " Gª¸¥g ¥ .[. 30 ⁄ ˜`¡A60 ⁄ ˜`⁄¸120 ⁄ ˜`«Æ "⁄† ›Mƒ s ‹ †v S‹ 96%¡A89%⁄¸ 47%¡Cƒ ƒ„„ ' ... ¥ S†ŒG†Z⁄† ›M ˘ ‚ ¡AS ›»{ 60 ⁄ ˜`‹ ł A•"⁄ fi ¶¡¡C

„ ' EGF ⁄S† Bz¡A«h¥ ¥H Lƒ †M⁄S i†G i 36 ⁄pfi «Æ¡AƒA¥[⁄J 200 ng/ml ⁄S EGF ' 37 ¢ JA 5%CO2-95% air ⁄ ⁄ B†Ł0 ⁄ ˜`¡AƒA –N † ›M ¥H 2mM ethylenediaminetetraacetic acid ¡]EDTA¡ ^ »†ŒG Bz¤ ˘ ¤ ›Ø¡A¤ ¤^¥HTrypan Blue ‹ ¥ ¡A¤^ƒb¯ ª Ł^Ł ⁄ U ‛„¤ ƒ s ‹ †v¸› p˘¡C † " Gª¸¥g ¥ .[. 30 ⁄ ˜`¡A60 ⁄ ˜`⁄¸120 ⁄ ˜`«Æ "⁄† ›Mƒ s ‹ †v S‹ 96%¡A89%⁄¸ 47%¡Cƒ ƒ„„ ' ... ¥ S†ŒG†Z⁄† ›M ˘ ‚ ¡AS ›»{ 60 ⁄ ˜`‹ ł A•"⁄ fi ¶¡¡C

⁄W› z ƒ U "–Ł¥Bz«Æ"⁄† ›M• » †G¥H1.5 † @ ¡A0.125M Tris buffer, pH 6.8, 2% ¡]w/v ¡^SDS, 5% ¡]v/v ¡^ 2-mercaptoethanol ¡A10% ¡] w/v ¡^ glycerol¡A0.001%¡]w/v ¡^bromophenol blue "⁄ Laemmli sample solution [8]• » , ¡A¥ .[. 100¢ JA⁄T⁄ ˜`¡AƒA, g„ ¶W " ł Ł⁄100,000 g¡A4¢J⁄S⁄U⁄@⁄p fi ¡Aƒ ¶ ⁄W†M†G¡C¥ –N⁄W†M†G' ¶Wq«Æ ¤œ 10 ?g "⁄¿ @ ¥HS @ sodium dodecyl sulfate-polyacrylamide gel ¡ÆkÆctrophoresis¡]SDS-PAGE¡^„ Œa⁄ "R [9]¡C¤^–N„ Œa⁄ "R«Æ⁄S† J ¥ ´ † ƒ nitrocellulose membrane ¡C ƒ„ nitrocellulose membrane ¥ ¥H 5% † fl ⁄ß¥⁄¥[⁄WTris buffer saline ⁄@⁄pfi ¤ "¡_]blocking¡« ÐM⁄@'°"⁄ †ƒX¡A ⁄S«Æ¥HPBS ¤ R~⁄Tƒ,¡AƒA¥H primary antibody¡]ƒp⁄W' ›z"⁄ƒ Ú S ¯Ø¡^S@ ¥¸⁄@⁄pfi ¡C¥H PBS ¤ R~⁄Tƒ,«Æ¡AƒA ¥H, g horseradish peroxidase †ƒX ¡]HRP-conjugated¡"⁄goat anti-mouse ' ¸anti-rat IgG S@¥¸⁄@⁄pfi ¡C¥H PBS ¤ R~ ⁄T ƒ, «Æ ¡A ¥H enhanced chemiluminescence¡]ECL; Amersham, Aylesbury, UK¡^⁄ Ł " ŕn¥œX ¥œ⁄ø ⁄W ¥ ¥X¤ ¡C

¥t¥~¡A' ... ¥S⁄J¸EGF ł Bz † S «Æ¦Aƒ ¶ † ›M i" ⁄W†M†G¤ ·œ'w ¥i•»' °E-cadherin¡C¥i•»' °E-cadherin ⁄S·œ¶q⁄ D ¤ ¥‹W'–` ÷o"⁄T...h ƒ ¡ »fl†s †S K ⁄ "RfiM¥ ¡]Zymed Laboratories Inc., San Francisco,

USA¡^¡A¤ ／／§tf¤mŏusf¡^«¡]– ""^ «Y¡]„ˇ／ GB„ˇ／T¡B„ˇ¥|¡^¡Cf
§ H¯Ø-cadherin／£ƒP¡]epitope¡^ ‹O• § › ¶i／@¤B·œ' w ¥ ₺»'°
" " ŧfi§ ¯Ø¡ C § Q ¥ ¸／s ;"„Lfiæ／E-cadherin »P／W¥ ¥˝"Ł¿ E fl – ¤Ø
¡]horseradish peroxidase, HRP¡^... »x ／§¶¡""^ «Yfi ¡A«o¥i o†{¥ i »'°
ƒ b ƒfl§ ¯Ø／W¡A¥ i M'／w HØ¥ i »'° E-cadherin "¿ @ ‰ TŒ•|¤ ¤ ... v̄T
E-cadherin ／§ @«¡Cfb" Ł 450nm ƒ·／¡]" ／@¡^¡CEl-Bahrawy [11]·£
fi¡A§ ¸ELISA ¯ " œ ¡A¥i¯ ¤œ¤ ¤ †˜／@ƒ "¥˝"Łƒ]fl –¤¯Ø»P¤ ¥L
ŧ ¥œ« ¡A¤^§ ¸" Ł 630nm § @ ‹`B "" ／ ／l §@ ¥¸¡A ,g ¥ tyrosine
¥¿C¤ C／@ › ... ¸¥ » ƒ ／ŧ"˝ ´ø›phosphorylation ƒ ... v̄Tcatenin "¥\
¯¸¡A¤ ^œ¤ ¥ › § ¡ › ‹ ¿œ¶qC fl¡C¥ ƒ ¥˝／˜ ／W"§ ¯ ' ¸ ƒ]‹ ¯
' ƒŧ"› G ø fl f – w ŧ ß • ／Ø¥W"§ ¯ ¡A£ƒŧ¥ifl ¶¡– x.¯catenin
ø ‹ ª ß ·[◀0] ' – ´¸"›G／`£ "¥ Ł ƒ ›P† ›M"／£¥¿–'¥˝"Ł／¸
／ŧN]¥ ›G／` £'ƒ¸¥ ›G／`£¡^¥H／¸ ／˜[12¡A13¡A14]¡CShiozakiS « ¥X¡A
†O／ †„Ł†M ŁN¤q¶Nifø†／´ŧN ／@ƒ ／W¥ ¥˝"Łƒ]fl –¤¯Ø¤ ¤ ¤°
／«D ø／§›G %ƒ¤q/œ／U¡C ƒŧ"...— ¿E¡A–N§ ¤ E-cadherin"˝" † ƒ ›P
¥ »£ƒbƒ ‹ ¶ « Æ « KO§0s'U¡A¡€"‰ ø† ›M"´ † ／¸« IS' °[12]¡Cƒ ƒb§
¤ ¤œ¥ Ł˜¥ ¡C › "„Œ †"G¯ª¥ ¡A¤ ¤ ¥˝"Łƒ]fl – ¤ ¯Ø"˝ŧ Bz«Æ¡A› Gø† ›M i／W†M†G
／"˝¥ i »'Ë-cadherin¿ @ ¡Aƒb SC-M1／¸AGS¤ ˝ † ›M– §et{ƒ•N
,q"˝› §C¡Cƒb› G ßf"˝ƒ †M／¡A,ß "˝¥ i »'Ë-cadherin¿ @ ˝ ¥ „w«Æ
／£¤¸¡A¥ §Mefŧø´ † "˝† ¶H ／£¤¸¡A¥ §Mefŧø´ † "˝† ¶H
alpha-catenin¡]„ˇ／G¡^¡Bbeta-catenin [15]¡CNU-GC-3† ›M" i／W†M†G／
¡]„ˇ／T¡^¡Bgamma-catenin¡]„ˇ¥|¡^«h "˝¥ i »'Ë-cadherin¥ ƒ·／ "˝`˝
L' œª／§¯ ／ ¡C ƒV¡A¥u«O« „F¤ ƒ•N§q"˝fi §O¡C†o † "G¯ª¥ ／W¥ ¥˝"Łż E fl – ¤Ø«O•|...v
Mƒ ¡A,g„L／W¥ ¥˝"Łż E fl – ¤ ¯T¤ ø† ›ME-cadherin"˝" †{¡A¥u«O
¯Ø"˝... v̄T／§／U¡A„ ƒ„／T" › Gø† ›MiŁ {« "˝／ jpƒ ／w¡C
¤ ·œ' w ¥ ₺»'°E-cadherin／§" †{¡A«o §››"« o†{ƒb› Gø† ›M／¸A
¥i o†{ E-cadherin "¿ @ ¥i¤ ¤ ...v E-cadherin"˝" †{•|¤ ¤ ／W¥ ¥˝"Łƒ]
¯Tƒ › §C¡]" ／@¡^¡C fl – ¤ ¯Ø"˝... v̄T¡Aƒ ¥B¥H¥ i »'°
E-cadherin"˝... v̄T† ¯ª ¡C¶i／@¤B"˝ ...Æ†M¡A» ›n„ ' catenin¡A／W¥ ¥˝"Łƒ]
fl –¤¯Ø¡Atyrosine phosphorylation¥H ／¸¥ i »'Ë-cadherin／§¶¡"˝‰ ˜Ł^ «Y
¶i／@¤B"˝– ·Q¡C

／›¡B Ł“G

› Gø† ›MiŁ„ g ... ¥ §J«ŧB／§z
E-cadherin¡Aƒbƒ Ł／Ł ¥´IS'¥kU¡ Æ†
' œª／§.. W j" †{／S† ¶H¡]„ˇ／@¡ ^Aƒ
alpha-catenin¡]„ˇ／G¡^¡Bbeta-catenin
¡]„ˇ／T¡^¡Bgamma-catenin¡]„ˇ¥|¡^«h
L' œª／§¯ ／ ¡C

Mƒ ¡A,g„L／W¥ ¥˝"Łż E fl – ¤ ¯Ø"˝... v̄T／§／U¡A„ ƒ„／T" › Gø† ›MiŁ ¤ ·œ' w ¥ ₺»'°E-cadherin／§" †{¡A«o ¥i o†{ E-cadherin "¿ @ ¥i¤ ¤ ...v ¯Tƒ › §C¡]" ／@¡^¡C

/»¡B Ł“G

Catenin‹OE-cadherin ' ŧs †"˝ ¥ Ł ' °ŧ J ¥‰ŁZƒ]ƒ„– ·œcatenin¥i¥H ... v̄TE-cadherin"˝" †{¡C Mƒ – q §› "˝" ¤B †"G¯ª¥ ¡Aƒb.. ¥§U"... v̄T／U ƒ ¥Gcatenin¤^¤Sƒŧ„ E-cadherinƒŧ"‰

/ C ¡ B ƒ ,Œfi˘

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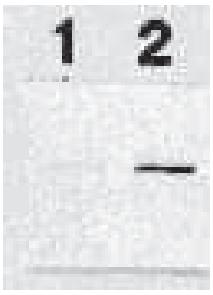
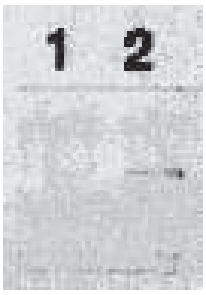
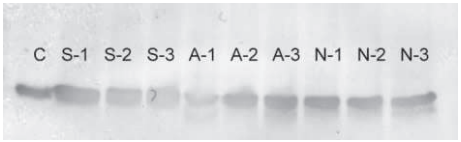
„ ˇ /@¡Bcadherinƒ b › G ø † ›MfiŁ“”
† {C1¡ G ¥ ¿ – ‘ ¡/C2¡G… ¥ §J¥H
«ÆC
¥“¡GSC-M1¡ Fk¡GNU-GC-3¡C

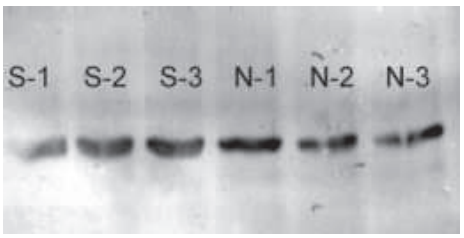




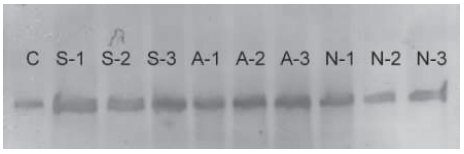
„˜¥|¡BGamma-cateninƒ b › G ø ›MfiŁ
“ ” “ †{¡CGcontrol¡AS:SC-M1¡AA¡G
AGS¡AN:NU-GC-3¡C1¡ G ¥ ¿ – ‘ ¡/C
2¡G… ¥§J0/ ˜ ¥ HÆC3¡G… ¥§J
60/ ˜ ¥ HÆC



„ˇ/G¡BAlpha-cateninƒ b › G ø ›MfiŁ
“ ” “ †{$¡SC-M1¡AN:NU-GC-3¡C1¡G
¥ ¿ – ‘ ¡/C2¡G… ¥§J0/ ˜ ¥H
«ÆC3¡G… ¥§J0/ ˜ ¥ HÆC



„ˇ/T¡BBeta-cateninƒ b › G ø †MfiŁ“”
“ †¡C¡Gcontrol¡AS:SC-M1¡AA¡G
AGS¡AN:NU-GC-3¡C1¡ G ¥ ¿ – ‘ ¡/C
2¡G… ¥§J0/ ˜ ¥ HÆC3¡G… ¥§J
60/ ˜ ¥ HÆC



“ @¡B G ø † › Mfi Ł , gL... ¥ §J‡B†z ¡] 42.5¢ ¡A30/ ˜ ¡A60/ ˜ ¡A120/ ˜ ¡ ªqB« ÆM/EGFR‡ ‰z«Æ ” ¡ / W† ‰GA¥ ¡ »'E-cadherin¿ @ (ng / ml)“”/ æß¡C

Treatment	SC-M1	NU-GC-3	AGS
37°C	215.4 ± 3.43	151.6 ± 3.49	94.2 ± 1.41
42.5°C, 30 min.	234.8 ± 2.94	164.4 ± 2.56	94.5 ± 0.92
p	0.014	0.106	0.824
42.5°C, 60 min.	396.5 ± 4.21	197.2 ± 1.26	93.8 ± 2.57
p	<0.001	0.001	0.902
42.5°C, 120 min.	329.4 ± 6.25	180.9 ± 1.92	92.3 ± 2.06
p	0.001	0.019	0.407
EGFR	168.0 ± 13.57	162.8 ± 2.16	65.1 ± 3.21
p	0.0315	0.0794	0.0139

p: »P37¢ J ⁄ § ⁄ Ðæ,ß¡C