Mitogen-activated Protein Kinases p38-beta Contributes to TNF-alpha Resistance in Oral Cancer Mediated BAD^{ser112} Phosphorylation

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Backgrounds: When oral cancer is diagnosed, the three-year survival rate prediction of this patient is only 58% and can only be increased to 74% after surgery in Taiwan. Before the squamous cell carcinoma (SCC) formed, hyperlosia is an initial stage symptom induced by EGFR over expression in a long term inflammation. Thus, tumor necrosis factor-alpha (TNF-α) releasing in inflammation lose it original anti-tumor function and a tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) resistance might happen in mane cases. In our pervious study indicated p38β MAPK over expression in oral cancer might be associated with TRAIL resistance through serine 122 of BAD phosphorylation, and which is a gatekeeper of BAD-mediated apoptosis.

Materials and Methods: In this research, a cell line T28 from 4-nitroquinoline-N-oxode (4-NQO) induced oral cancer in C57B mouse and human tongue squamous cell carcinoma cell line SCC4 were screened in this TNF-α resistance issue. All proteins from cell were analysis by immune blot assay.

Results: TNF-α releasing is through p38 mitogen-activated protein kinases and TNF-α resistance exists in both T28 and SCC4 cell line. Further, the serine 136 of BAD phosphorylation was promoted by p38α MAPK isoform and the serine 122 and 155 of BAD phosphorylation were promoted by p38β MAPK and also block the apoptosis cause by TNF-α. A p38β MAPK inhibitor SB202190 (10μM) was used, the cell cycle arrested at G2 phase from 9.5% to 17.36% within 24h treatment in SCC4 cells.

Conclusion: Over expression of p38β MAPK in oral cancer indeed caused TNF-α induced apoptosis resistance by BAD phosphorylation. And serine 122 of BAD is control by p38β MAPK. This suggests that p38β MAPK is a possible anticancer target in oral cancer therapy.

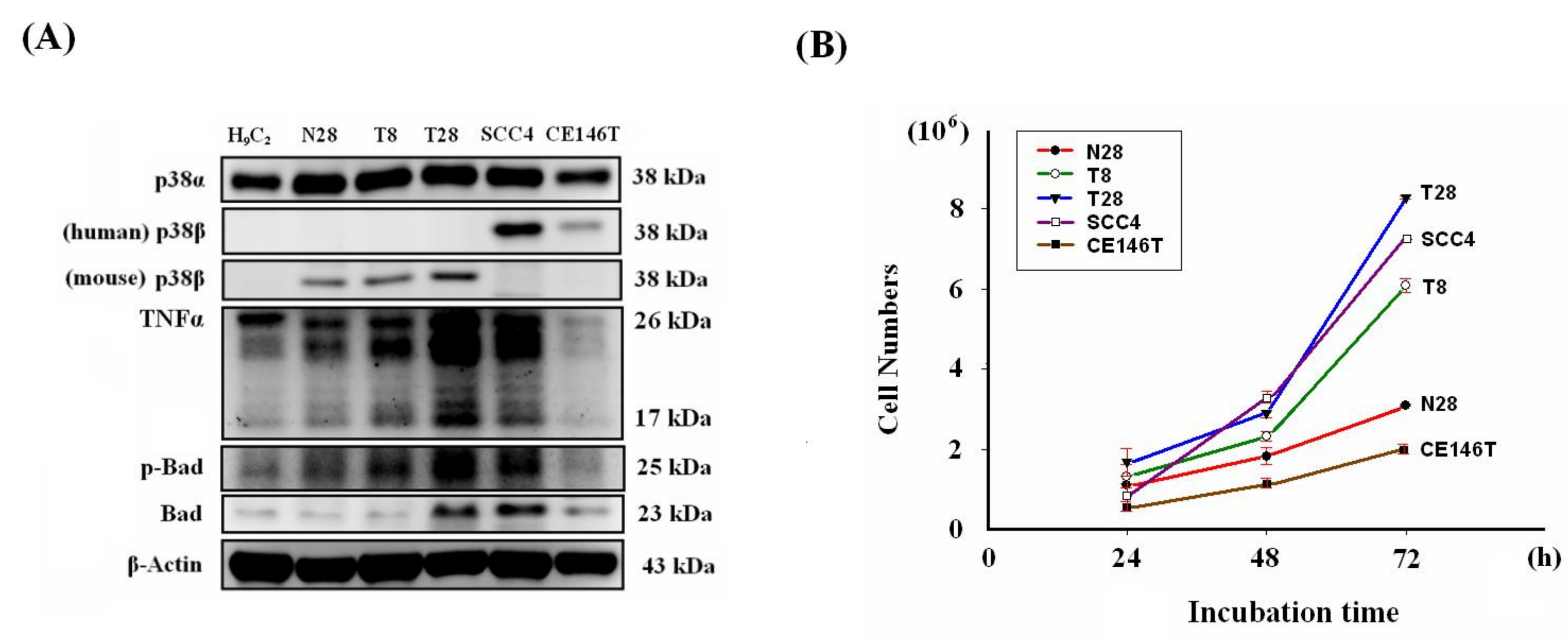

Figure 1. The relationships between TNF-α expressions and cell proliferation rate in normal / oral cancer cells. (A) p38 MAPK over expression induced TNF-α releasing and BAD phosphorylation in tumor cell lines rather than normal cells lines. (B) The cell proliferation rates in tumor cell lines are higher than normal cell lines. (Cell lines: H9C2 is a rat cardiomyoblast cell line as normal. N28 is none tumor cell line from C57B mouse. T8 and T28 are 4NQO induced oral cancer cell from C57B mouse, SCC4 and CE146T are human oral cancer cell lines.)

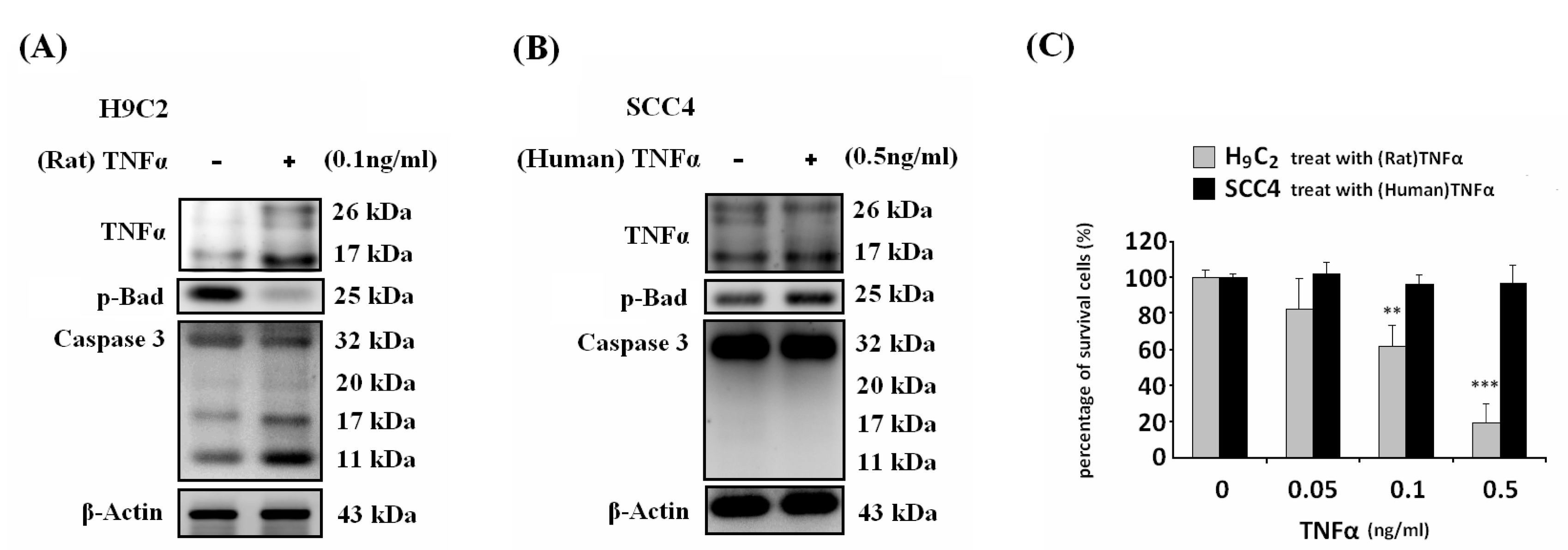

Figure 2. The role of phosphorylated BAD in TNF-α resistance. (A) After 24h 5ng/ml TNF-α treatment, Caspase 3 expression increased and phosphorylated BAD decreased in H9C2 cell. (B) The same 24 h 5ng/ml TNF-α treatment in SCC4 cell can not induce the BAD dependent apoptosis, and show the TNF-α resistance existed in this oral cancer cell line. (C) The cell survival percentage between H9C2 and SCC4 cell line in 24h TNF-α treatment. Data are expressed as mean ± SE (n=3) and * = p <0.01, ** = p <0.05, *** = p<0.001 as compared with the control group.

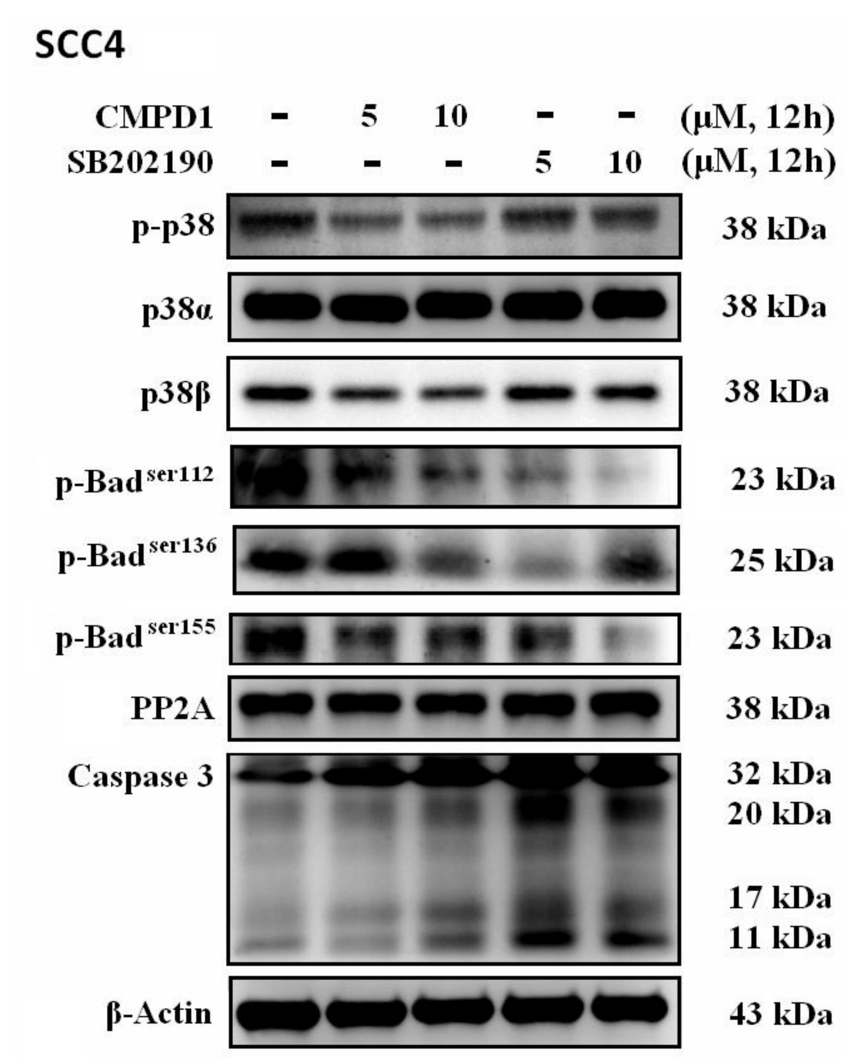

Figure 3. The p38α/βMAPK related BAD phosphorylation. The p38α/β MAPK specific inhibitor CMPD1(10μM) and SB202190(10μM) were used to block the p38α/β expressions in human oral cancer cell line SCC4 for 24h, and the down stream BAD phosphorylation location (serine 112, 136, 155) decreased follow the differ p38α/β inhibitions. The SB202190 treatments remarkable lowered the p-BAD^{ser112} and p-BAD^{ser155} induced the caspase 3 cleavage in SCC4 cells.

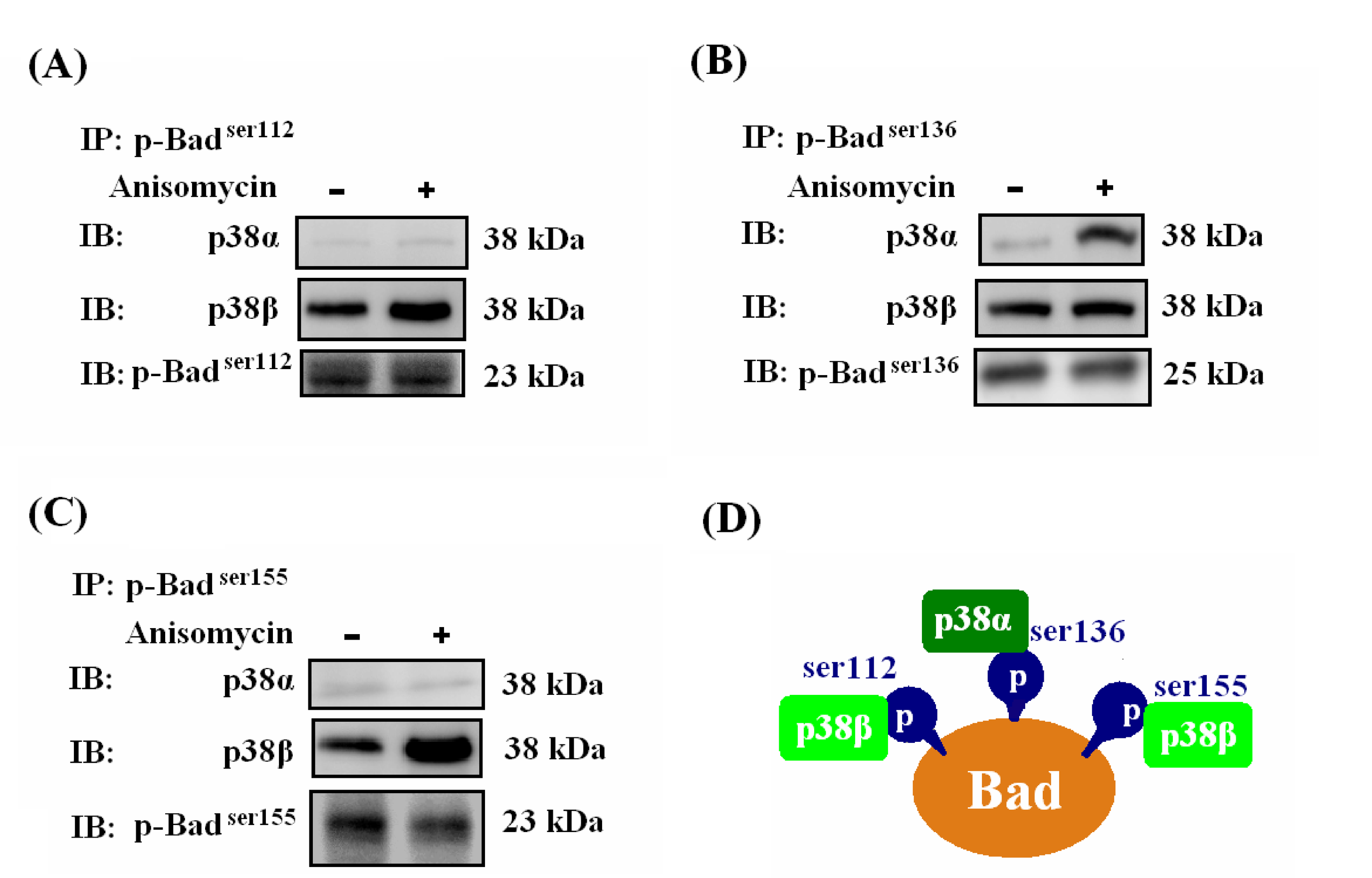

Figure 4. The complex formed by p38α/βMAPK and different BAD phosphorylation locations. After 30 min anisomycin treatments, the co-immunoprecipitation assay was used in complex detection between p38α/βMAPK and differ BAD phosphorylation location (serine 112, 136, 155). The result shown that, p38α combined with p-BAD ^{ser155} only and p38β combined with p-BAD^{ser112} and p-BAD^{ser155} in the formed complex.

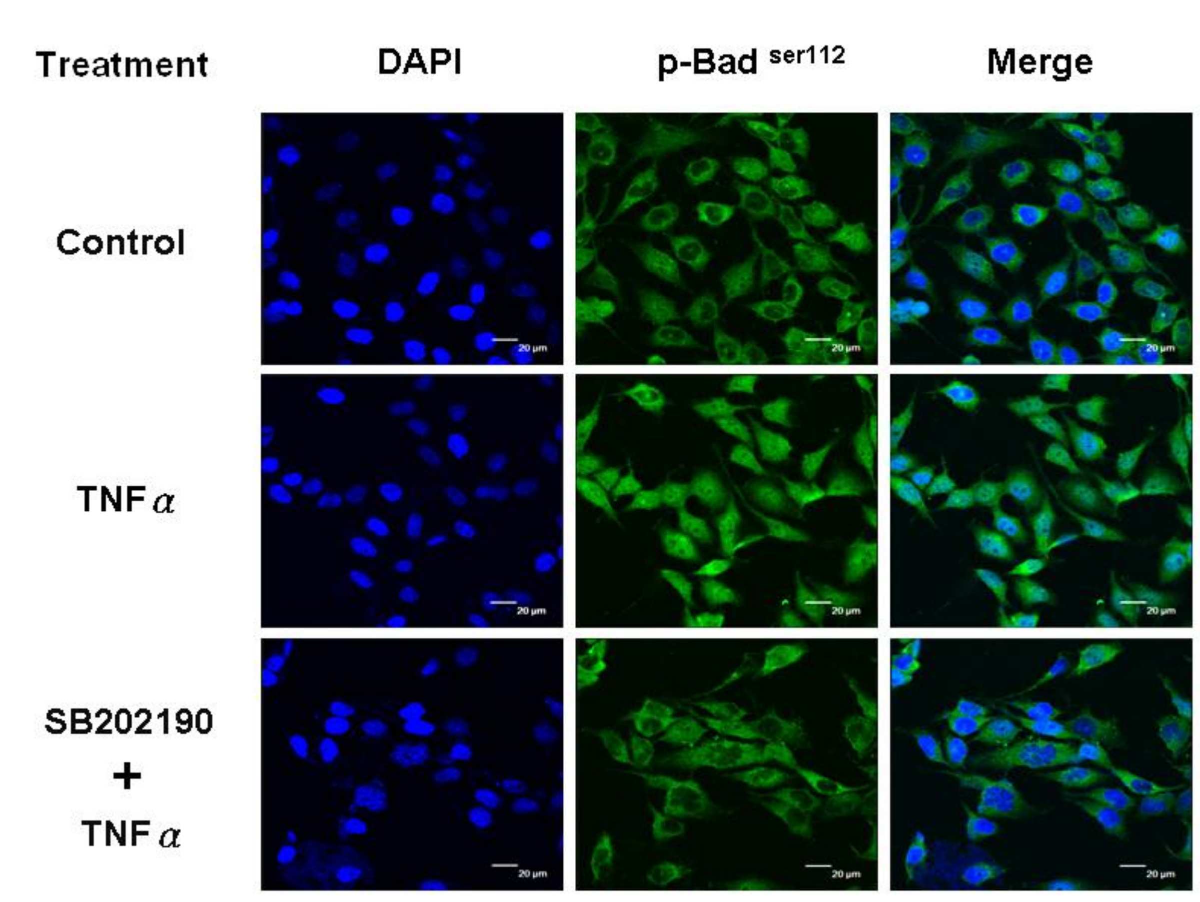

Figure 5. The SB202190 inhibit the p-BADser112 expressions in TNF-α resistance oral cancer cells. After 24h TNF-α (human, 5ng) or SB202190 (10μM) co-treatments, the Laser Scanning Confocal Microscope imagination shown a partial p-BAD^{ser112} exist in cytoplasm (in degradation progress) and a partial p-BAD^{ser112} exist in nucleus. After TNF-α treatment for 24h, a large mount of p-BAD^{ser112} exist in nucleus. SB202190 (10μM) and TNF-α co-treatments for 24h, p-BAD^{ser112} exist in cytoplasm only.

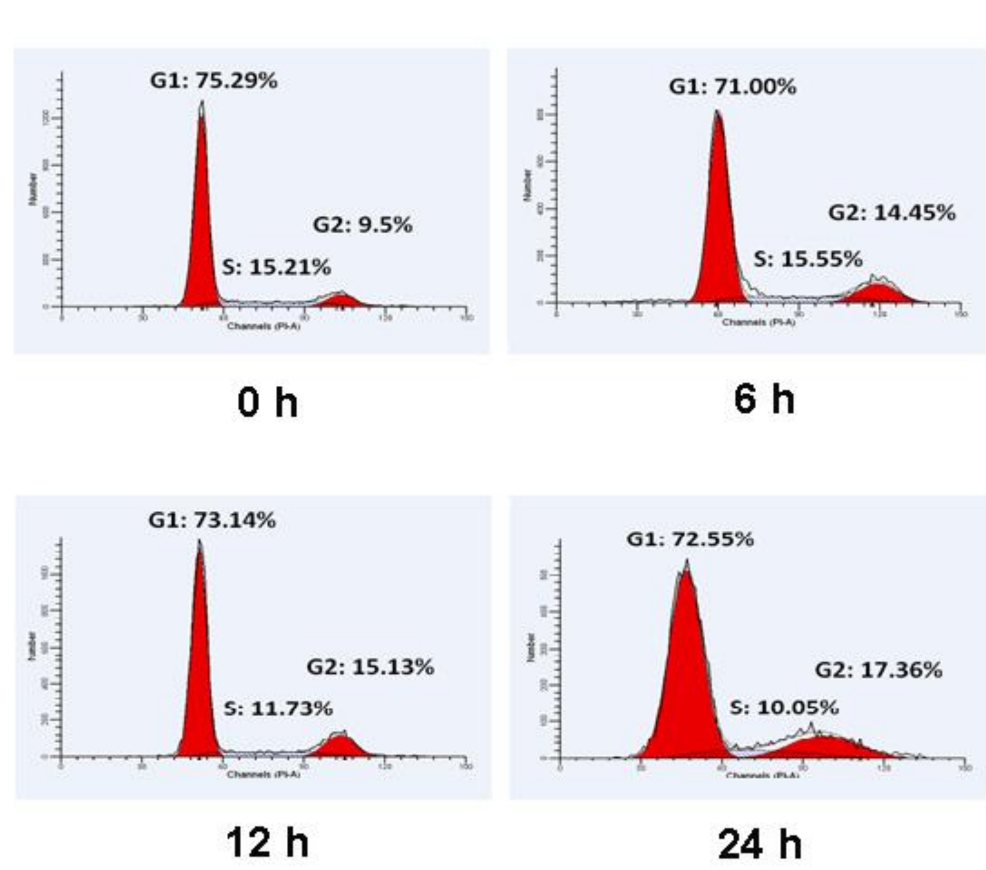

Figure 6. The SB202190 treatments caused a cell cycle arrest in SCC4 cells. The cell cycle of human oral cancer cell SCC4 arrest while A p38β specific inhibitor SB202190 (10μM) treatments from 0 to 24h. The cell cycle of SCC4 cell is major arrest at G2/M, from 9.50% (control) to 17.36% (SB202190, 10μM) within 24h.

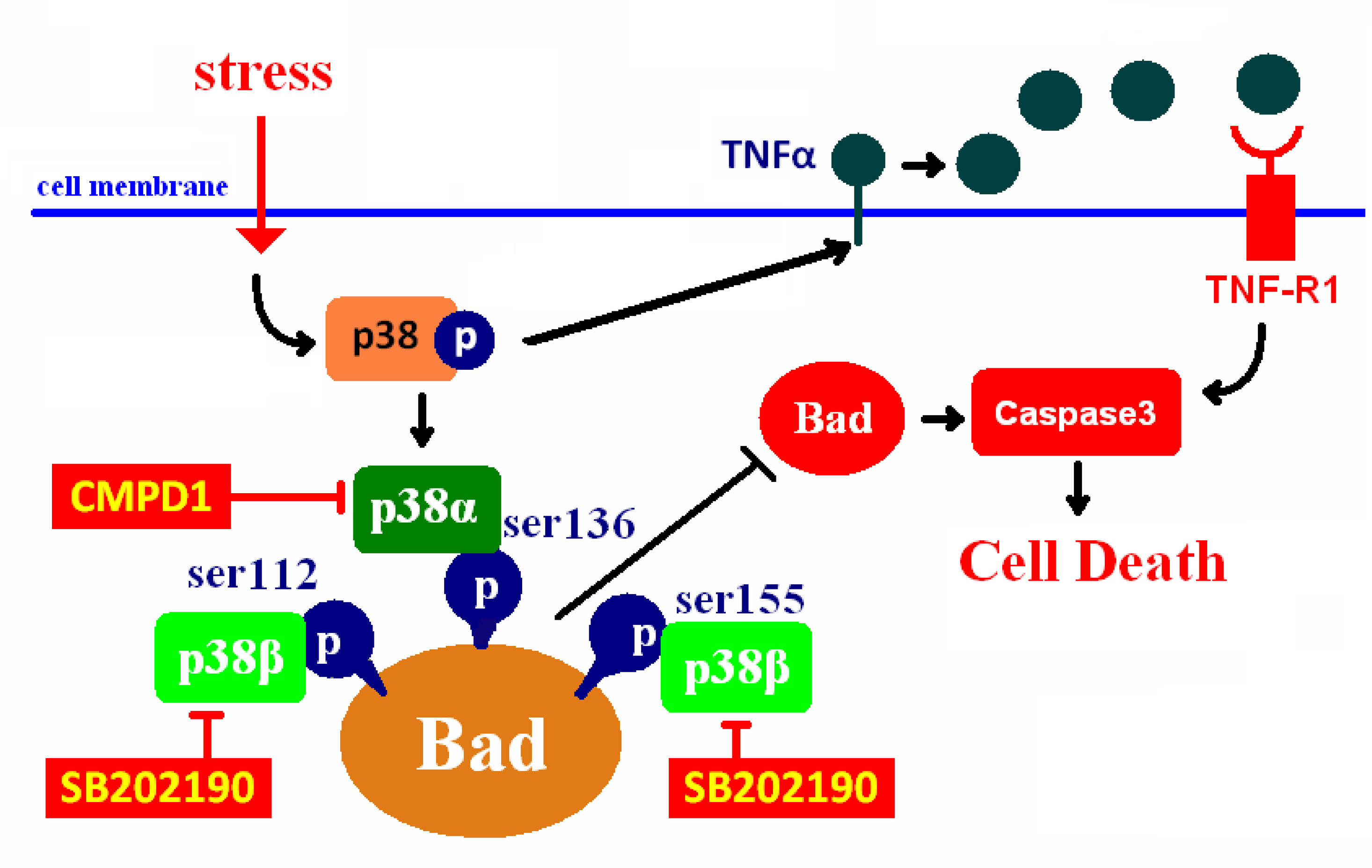

Figure 7. The p38 MAPK dominate TNF-α release and p38β MAPK expression cause a TNF-α resistance through the downstream BAD phosphorylation. In several published research shown that the TNF-α releasing is dominated by p38 MAPK (p38α major) activation by the outcome stress. At the same time, the stress could induce the p38α MAPK provides a BAD phosphorylation at serine 155 and p38β MAPK expression provides a BAD phosphorylation at serine 112 and 155. The serine 112 of BAD plays a gate keeper role prevent BAD dependent apoptosis caused by the protein phosphatases (such as PP2A) dephosphorylation. Finally, the BAD phosphorylation provide a protection of human oral cancer cell from the stress caused TNF-α induce apoptosis and promotes oral cancer a TNF-α resistance ability.