肿瘤热化疗中化疗药物的选择

彭 楠 马爱敏
(解放军总医院 100853)

摘要 目的:肿瘤治疗进入综合治疗时代,临床已应用热疗联合化疗和/或放疗治疗多种肿瘤,但热化疗中还有许多问题需要明确,如最佳的温度和作用时间,热化疗是药物的选择等。方法:通过总结许多国内外的基础试验研究和临床应用结果,评价肿瘤热化疗的协同作用。结果:体外细胞试验表明化疗药物的毒性与治疗温度和时间相关,温度37.0~41.0℃(41.5℃)合并药物时,细胞生存率急剧下降,而除ADR外,41.0~43.0℃时细胞生存率下降不明显。体内实验表明9种药物中,除BLM外热增长比例(TER)在41.5℃和43.0℃无明显区别,其中马法兰的TER最高。热化疗的临床研究还处于初期,但用马法兰和TNF进行隔离肢体热灌注治疗黑色素瘤和软组织肉瘤效果确切。结论:热疗联合化疗有协同抗肿瘤作用,应进一步深入研究。

关键词:肿瘤治疗;热化疗;化疗药物;细胞生存率

Selection of Chemotherapeutics in Thermochemotherapy of Tumor

PENG Nan, MA Ai-min
Department of Physiotherapy, General Hospital of Liberation Army, Wukesong 100853, Beijing, China

ABSTRACT Objective: To discuss the optimal temperature(OT) and action time(AT), selection of chemotherapeutics in the thermochemotherapy(TCT) of tumor. **Methods:** The synergistic action in the TCT of tumor was evaluated by summarizing the basal experimental investigation and clinical application of the internal and external. **Results:** Exosomatic cell test showed that the toxicity of chemotherapeutics was relative to treating temperature and time. When the temperature was 37.0-41.00C(41.50C) with medication, cell survival rates decreased suddenly except Adr; the test in vivo showed that out of the 9 drugs, L-Sarcolysinum's(L-PAM) TER(=Thermal Enhancement Ratio) was the highest at 41.50C and 43.00C, except BLM without significant difference. Although the clinical study on TCT is still in the initial stage, hyperthermic isolated limb perfusion with L-PAM and TNF in the treatment of melanoma and soft-tissue sarcoma has got good effects. **Conclusion:** Thermotherapy combined with chemotherapy has synergistic action against tumor, which should be further studied.

Key words; Treating tumor; Thermochemotherapy(TCT); Chemotherapeutics; Cell survival rate

目前肿瘤治疗已经进入综合治疗的时代,肿瘤热疗是继手术、放疗、化疗、免疫治疗后的新手段,而且随着生物医学工程和电子技术的迅猛发展,肿瘤热疗也有了长足的进步,从最初的水浴、热毯包裹加热,到现在的射频、微波、高强度聚焦超声等加热方式,治疗也由原来的局部或全身加热,到今天的组织间定向损毁。临床应用也发现热疗与放、化疗均有较好的协同抗肿瘤作用,而肿瘤热化疗时还有许多问题不是十分清楚,如最适合的温度范围和加温时间、热化疗时什么药物最有效等。

1 实验研究

1.1 治疗温度和治疗时间

热化疗实验的主要目的之一是揭示热提高化疗药物的细胞毒性在多大程度依靠治疗温度和时间,即化疗药物的细胞毒性与治疗温度和时间的相关性。荷直径4mm FSa-Ⅱ肿瘤(肿瘤体积约35mm3)的动物接受不同剂量环磷酰胺(CY),然后立即进行局部水浴加温,温度范围37~44.5℃ 30min,仅有一组动物为室温下接受药物处理,肿瘤温度是28℃,观察平均肿瘤生长时间(TG:平均TG时间要求从治疗之日计算,50%的肿瘤增至1000mm3的时间)[1,2]。以TG为纵坐标,CY是为横坐标,作肿瘤反应曲线符合线性回归,当治疗温度从室温升至37℃,进一步至40.5℃时,热增长比例(TER Thermal enhancement ratio)随温度的升高而升,40.5℃时TER为2.54;40.5~42.5℃时TER较固定,当温度进一步升至43.5~44.5℃,TER轻度增加,从2.74到2.96。42.5℃以下和43.5℃以上热效应明显不同。< 42.5℃ 30min不延长TG,但加温至43.5~44.5℃ 30min,TG延长。这一结果表明大量热增长是在加温至40.5℃时获得,治疗进一步升至40.5~44.5℃时,与TER增大可能无关,因为42.5℃以上治疗的升高是热致死所造成的结果。

用CDDP作用荷乳腺癌的C3H小鼠,每只腹腔注射CDDP 0~8mg/kg,15min后加热至40.5~43.5℃ 60min。以TG和CDDP剂量作剂量效应曲线。当温度从41.0℃升至41.5℃时,TER从1.3增至2.9。温度进一步升到42.5℃或43.5℃时,TER没有进一步增加。41.5℃和43.5℃时热化疗效果的不同在于43.5℃时有高热抗癌作用,而41.5℃时没有细胞毒作用。获得最大热增长的最低温度是41.5℃,比40.5℃结果高1℃。这一区别可能的原因是肿瘤的类型、大小、药物和/或肿瘤内温度表达方式的不同所致。

肿瘤加热的实验结果表明,亚高温(39.5~41.5℃)可以增加肿瘤组织内的血流,可是过度的加热可能降低肿瘤的血流,研究表明在43℃以下轻微热疗能明显增加某些化疗药物细胞毒性[4,5]。最低的组织温度(≤~41℃)是可以达到的,血液增加可能有助增加组织中药物浓度。可是,某些药物的细胞毒性与组织pH值,氧分压,其他微循环因素有关。药物在组织中的分布也不均一。

研究中加热时间均用30min,这是由于加热超过30分钟并不能明显增加肿瘤的反应[2],这归因于化疗药物在组织中

作者简介:彭楠,(1964-),女,副主任医师,硕士研究生导师,在读博士。E-mail: pengnan66@Sohu.com

(收稿日期:2006-02-21 接受日期:2006-04-10)

的代谢。小鼠腹腔注射 CY, 其活性分子在鼠中半衰期为 20min, 这意味着为了获得有效的TER, CY 给药后立即加热, 超过20min 作用几乎不增加[6]。CY 可能是血浆半衰期长的化疗药物之一, 因为它需要体内激活, 其他化疗药不需要体内激活, 诸如 CDDP 可能血浆半衰期比 CY 短得多[7]。因此, 许多化疗药物被单一应用于腹腔注射, 而不是持续灌注, 小鼠加热 30min, 足够产生有效的 TER。可是, 药物仍保留在肿瘤组织中, 加热超过 30min 可能产生残余作用。

1.2 化疗药物的热增强效应

1.2.1 体外实验: 国外学者用进行了六种药物的体外实验, 观察对细胞生存曲线的影响。总之化疗加热疗后细胞生存曲线的斜率急剧下降。温度从 37.0℃升至41.0℃(41.5℃)后, 生存率下降明显的药物有 BCNU、CDDP、BLM[8,13], 而 MMC、5- FU 影响小[9], 只有 ADR 在 41.0~ 43.0℃时生存率下降明显[10]。

CDDP、BLM、5- FU 活化能折点温度在 41.0~ 41.5℃, 可是没有发现 MMC 和 BCNU 在 37.0~ 43.0℃之间有折点。有趣的是 CDDP 和 5- FU 的活化能与单纯热疗(41.0~ 43.5℃或 43.5℃)一样, 均为 200±10kcol/mol, 而 BLM 活化能比单纯热疗(41.0±43.0℃或 43.5℃)稍低[(165±15) kcal/mol]。低于 41.0℃(或 41.5℃)时, BCNU 活化能最大, 5- FU 最小, CDDP 作用 CHO 细胞时活化能在 43℃以上获得, 但活化能与单纯热疗一样≤140kcal/mol[11]。这些结果表明热增加许多化疗药物的细胞毒性作用在 37.0℃~ 41.0(41.5℃)时最明显, 此时 TER 达最大值, 在 41.0(41.5)~ 43.0(43.5)℃之间不变。< 43.5℃时的热增强作用很可能是热细胞毒性的附加作用。

国外学者对 20 种人肿瘤细胞株进行系统研究, 分析热化疗的协同作用, 这些细胞株包括神经母细胞瘤、艾文氏瘤、胶质细胞瘤(GCT)、骨肉瘤, 化疗药物用 CDDP 5mg/ml 和 VP16 10mg/ml, 热处理 42℃或 43℃, 1 小时, 42℃与化疗药物联合应用作用增强 1.5 到 2.5 倍, 43℃与化疗合用时, 作用增强 2.6~ 14 倍[33]。

1.2.2 体内实验: 热化疗联合治疗荷4mmFSa- Ⅱ小鼠的抗肿瘤作用[12,14], 加热温度分室温(37℃)、41.5℃、43.5℃(除 MMC 仅在 41.5℃)。药物有 6 种: BCNU、CDDP、MMC、BLM、5- FU、ADR。BCNU、CDDP、BLM 三药的剂量效应曲线 41.5℃较 37℃急剧上升, 但其它三药 MMC, 5- FU, ADR 剂量效应曲线在 41.5℃时没有这种急剧升高现象。值得注意的是所有五种药物(BCNU、CDDP、BLM、5- FU、ADR)的剂量效应曲线的斜率在 43.5℃与 41.5℃时无明显不同。

在 9 种药物中, 烷化剂马法兰 L- PAM 的 TER 最大, 为 3.60±0.47; 其次是 CY 2.28±0.32; BCNU 2.27±0.23。另一烷化剂异环磷酰胺 IFO 在加热至 90min 时, TER 达 3.60±0.50, 与 L- PAM 接近。这可能是由于 IFO 活性物的血浆半衰期长(60min)所致, 其半衰期几乎是 CY 活性物血浆半衰期的三倍[15,16]。MMC、5- FU、ADR 三药接近一致。研究显示 Methotrexate and 无热增效作用[17]。

另一重要发现是 41.5℃与 43.5℃的TERs无明显区别, 但 BLM 例外, 在 41.5℃和 43.5℃时 TER 有明显不同。体内实验显示 BCNU 的 TER 最大, 体外实验也表明其在 37~ 41℃间活化能最大。体内实验 5- FU 的 TER 最小, 为 1.0, 体外实验其活化能也最低[18]。以 37~ 41℃(41.5℃)时的活化能和 TER(41.5℃/RT)作图, 显示活化能与体内 TER 有良好的关系, 尽管无线性关系, 但活化能大时, TER 也大。

1.3 生理温度及加热时的药物选择

比较在生理温度下给药的肿瘤最有效, 是否在加热时也最有效。生理温度下 9 种药物治疗 FSa- Ⅱ肿瘤, 3 种药物 TGD 时间大于 8 天, IFO 的TGD 最长, 达 9.5±1.6 天, 其次是 5- FU 8.7±0.5 天, CDDP 8.0±0.6 天, 因此, 在 37℃时适合选用 IFO、5- FU、CDDP, 41.5℃时最有效的药物是 L- PAM, 其 TGD 时间达 19.5±0.7 天, 其次是 IFO 14.2±1.0 天, CY 14.0±0.9 天[15,16]。

L- PAM 对 FSa- Ⅱ肿瘤在轻度加热时抗肿瘤作用增强最明显[14], 从 37℃ TGD 为 5.5±0.2 天, 增至 41.5℃时的 TGD 19.5±0.7 天, 这些结果表明生理温度下的药物选择不一定适合加热下应用。L- PAM 对 FSa- Ⅱ在轻度加热时抗肿瘤作用提高最明显, 但还不能肯定在加热时(41.5℃)对其他类型肿瘤也有同样的效果。

有文献报道 CDDP 用于抗 BT4A 大鼠肿瘤时[19], TER 最高为 4.96(Malla, 1985)。这个值比 CDDP 作用抗 FSa- Ⅱ肿瘤时 TER(1.59)高得多, 说明不同肿瘤的敏感性和热增效作用存在很大差异。有报道 CY 和 BCNU 的 TER, 在抗 RTF- 1 肿瘤时, BCNU 的 TER 较 CY 的大, 但在抗 FSa- Ⅱ肿瘤时 CY 的TER 更大些, 这也说明不同肿瘤间的差异[20]。BLM 用于鳞癌时效果大大提高[21]。这些结果鼓励探寻在加热下成功用于临床热化疗的有效药物。

2 临床用药

目前热化疗的临床研究还处于初期, 只有四肢黑色素瘤和软组织肉瘤的隔离热灌注化疗可能是例外。

高热隔离肢体灌注(HILP)合并化疗药物, 当血液温度达 43.3℃开始灌注, 外部加热是用热毯包裹肢体, 当皮肤肌肉温度达 38.3~ 40.0℃时, 将 L- PAM 60mg 注入灌注液, 灌注持续 90min。尽管这些病人仅接受 1 次 HILP, 70 例Ⅰ期病人 5 年生存率 86.3%, 30 例ⅢA 期达 70.4%, 而单纯 L- PAM 灌注的 27 例病人为 22.2%(回顾性对照), 高热隔离肢体灌注最大优势在于能保留肢体, 主要并发症有 7 例, 2 例死于肾衰和肺栓塞(肺梗塞), 5 例出现组织坏死[22]。分析这些合并症可能与组织温度太高(42℃)有关, 其他学者已经用 38.5~ 40℃低灌注温度。

由于过去 30~ 40 年, 相同的治疗不仅用于局部原发黑色素瘤(Ⅰ期)的病人, 而且用于那些局部复发(Ⅱ期)或局部转移(Ⅲ期)的病人。尽管药物剂量已经被调整, 主要的化疗药物是 L- PAM[23]。在同一机构也常使用 CDDP, 7 例曾用 CDDP(20~ 30mg/每升肢体体积)进行 HILP 治疗的患者复发率高(83%), 所有患者发生感觉神经或运动- 感觉神经疾病, 但没有全身毒性。广泛的神经疾病证明将 CDDP 用于 HILP 不恰当。

近年临床试验研究 L- PAM 加用肿瘤坏死(TNF)和干扰素(INF)或三药联合的作用[25], 阐明在过去临床化疗的经验中 TNF 与化疗药物有协同作用, 用 10 倍于全身给药量的 TNF 取得良好的效果, 而无明显增加全身毒性作用。这提供合理选择TNF(2~ 4mg)在HILP中用量, 大多数试验 L- PAM 标准剂量为 65~ 100mg[26]。Fraker 研究显示 4~ 6mgTNF 合并 IFN, L- PAM 治疗, CR、PR 分别为 76%(19/26)、36%(4/12)[27]。

L- PAM(melphalan)是治疗肢体黑色素瘤首选药物, 目前

用其他药物替代L-PAM是不恰当的,因为苯基丙氨酸是合成黑色素的前体,专门结合入黑色素细胞;其次没有其他药物用于HILP时CR超过L-PAM。再者,体外试验表明在9种被试验药中,L-PAM不仅TER最大,而且抗肿瘤的热增效作用也最大。

术中盆腔灌注治疗Duke's D期结肠癌,灌注液包含MMC 40μg/ml,加热45℃ 90min,热疗后局部复发率由未热疗的37%(13/35)降至11%(3/27)[28]。隔离灌注合并外部高热用于治疗肝转移癌。用肝动脉灌注化疗(5-FU 1000mg/m2和卡铂100mg)合并经体表热疗治疗9例肝转移癌,与8例单纯肝动脉灌注化疗比较,热化疗组和单纯化疗组PR分别为44%、25%[29]。

由美国9家医疗机构完成的用磁诱导装置治疗960例病人临床试验报告(Storm et al 1985),其中405人接受热化疗,结果热化疗肿瘤反应率比单纯热疗好,特别是在肿瘤内温度在40~42.9℃[30]。三组相互独立的研究小组改进了对组织的局部加热技术,治疗食管癌病人,热疗前口服博莱霉素,治疗每周1次,共6周,同时静脉注射CDDP每2周3次。接受这种治疗与单纯化疗ORR分别是30(6/20)%,和25(5/20),但切除组织学检查有效率热化组41(7/17)和19(3/16)%。

Colombo et al(1995, 1996)经三腔型导尿管放置微波(天线)加热器,能均匀加热膀胱壁,用60ml溶液含MMC(60mg)灌注膀胱,44例移行细胞癌(6例原发,38例复发肿瘤),热疗8次,每周2次,膀胱温度42.5~44.5℃,40min。CR(31/44),CR70%(31/44),其中原发肿瘤CR为100%[31]。随后随机试验(Colombo et al 1996)用含MMC 40mg的50ml溶液灌注,并加热至42.5~46℃,29例膀胱内灌注合并热疗,23例单纯膀胱内灌注化疗,两组CR分别是66%(16/29)和22%(5/23)。但两组生存率无明显差异[32]。

综上所述,许多研究表明热疗与化疗联合使用有明显的增效作用。这种增效作用的机制还有待深入研究,可能与热疗逆转肿瘤多药耐药性有关[34]。最近在研究耐药性的发生与细胞凋亡的关系中发现,热疗可以增加膜鞘磷脂水解,同时还可诱发酰基鞘氨醇的合成,为热疗逆转耐药提供了新的理论依据[35]。也有发现在MDR1启动子上存在热休克反应元件[36]。肿瘤热化疗中还有许多问题有待进一步研究[37]。

参考文献

[1] Urano M, Kim MS, Kahn J, et al. Effect of thermochemotherapy (combined cyclophosphamide and hyperthermia) given at various temperatures with or without glucose administration on a murine fibrosarcom[J]. Cancer Res, 1995, 45: 4162-4166

[2] Urano M, Kim MS. The effect of hyperglycemia on thermochemotherapy of a spontaneous murine fibrosarcoma[J]. Cancer Res, 1993, 43: 3041-3044

[3] Lindegaard J, Radacic M, Khalil AA, et al. Cisplatin and hyperthermia treatment of a C3H mammary carcinoma in vivo[J]. Acta oncologica, 1992, 31: 347-351

[4] Song CW, Shakil A, Osborn JG, et al. Tumor oxygenation is increased by hyperthermia at mild temperatures. Int J Hyperthermia, 1996, 12: 367-373

[5] Horsman MR, Overgaard J. Can mild hyperthermia improve tumor Oxygenation? [J] Int J Hyperthermia. 1997, 13: 141-147

[6] Begg AC, Smith KA. A bioassay for cyclophosphamide in blood, lung, and tumour[J]. British J Cancer. 1984, 49: 49-55

[7] Litterst CL. Plasma pharmacokinetics, urinary excretion, and tissue distribution of platinum following iv administration of cyclobutanedicarboxyatoplatinum-Ⅱ and cis-platin, to cis-platinum to rabbits[M]. Platinum Coordination Complexes in Cancer Chemotherapy, 1994: 71-81

[8] Urano M, Gerweck LE, Epstein R, et al. Response of a spontaneous murine tumor to hyperthermia: Factors which modify the thermal response in vivo[J]. Radiation Res, 1990, 83: 312-322

[9] Durse L, Covey JM, Collins C, et al. DNA damage, cytotoxicity and free radical formation by mitomycin C in human cells[J]. Chemical Biological Interaction, 1989, 71: 63-78

[10] Herman TS, Teicher BA, Chan V, et al. Effect of hyperthermia on the action of cis-diamminedichloroplat(Ⅱ), Rhodamine 1232[tetrachloroplatinum(Ⅱ)], Rhodamine 123, and potassium etrachloroplatinate in vitro and in vivo[j]. Cancer Res, 1988a, 48: 2335-2341

[11] Urano M, Kahn J, Majima H, et al. The cytotoxic effect of cis-diamminedichroloplatinum(Ⅱ) on cultured Chinese Hamster cells at elevated temperatures[J]. Int J Hyperthermia, 2000b, 6: 581-590

[12] Kaijser GP, Beijnen JH, Buit A, et al. Ifosfamide metabolism and pharmacokinetics[J]. Anticancer Res, 1994, 14: 517-532

[13] Klostergaard J, Leroux E, Siddik ZH, et al. Enhanced sensitivity of human colon tumor cell in vitro in response to thermochemoimmunotherapy[J]. Cancer Res, 1992, 52: 5271-5277

[14] Kuroda M, Urano M. Thermal enhancement of the effect of ifosfamide against a spontaneous murine fibrosarcoma, FSa-Ⅱ[J]. Int J Hyperthermia, 1997, 13: 124-131

[15] Wiedemann GJ, Oleire F, Knop E, et al. Ifosfamide and caroplatine combined with 41.8℃ whole-body hyperhtermia in patients with refractory sarcoma and malignant teratoma[J]. Cancer Res, 1994, 54: 5346-5350

[16] Wiedemann GJ, Siemens HJ, Mentzel M, et al. Effect of temperature on the theapeutic efficacy and pharmacokinatics of ifosfamide[J]. Cancer Res, 1993, 53: 4268-4272

[17] Monge OR, Rofstad EK, Kaalhus O. Thermochemotherapy in vivo of a C3H mouse mammary carcinoma: Single fraction heat and drug treatment[J]. European J Cancer, 1998, 24: 1661-1669

[18] Urano M. Thermochemotherapy: from in vitro and in vivo experiments to potential clinical application[M]. Hyperthermia and Oncology, 1994, 4, edited by M Urano and E Douple (VSP), pp. 169-204

[19] Mella O. Combined hyperthermia and cis-diamminedichloroplatinum in BD IX ratswith transplanted BF4A tumors[J]. Int J Hyperthermia, 1995, 1: 171-184

[20] Honess DJ, Bleehen NM. Sensitivity of normal mouse marrow and RIF-1 tumor to hyperthermia combined with cyclophosphamide or BCNU: A lack of therapeutic gain[J]. British J Cancer. 1982, 46: 236-248

[21] Hassanzadeh M, Chapman IV. Thermal enhancement of bleomycin-induced growth delay in a squamous carcinoma of CBA/HT mouse[J]. European J Cancer Clinical Oncology. 1982, 18: 795-797

[22] Stehlin Js, Giovanella BS, DE Ioplyi P, et al. Eleven years' experience with hyperthermic perfusion for melanoma of the extremities[J]. Cancer Res. 1979, 39: 2255-2257

[23] Alexander HR, Fraker DL, Bartlett DL. Isolated limb perfusion for malignant malanoma[J]. Seminars in Surgical Oncology. 1996, 12: 416-428

[24] Hajarizadeh H, Mueller CR, Woltering EA, et al. Phase Ⅰ-Ⅱ trial of hyperthermic isolated limb perfusion with cisplatin in the treatment of high risk malignant melanoma of the extremities[J]. Melanoma Research, 1991, 1: 55-61

(下转第21页)

参 考 文 献

[1] Garcia-Higuera I., Fenoglio J., Li Y., et al: Folding of proteins with WD-repeats: comparison of six members of the WD-repeat superfamily tothe G protein beta subunit[J]. Biochemistry 1996,35:13985-13994

[2] Smith T. F., Gaitatzes C., Saxena K. et al: The WD repeat: a common architecture for diverse functions[J]. Trends Biochem. Sci. 1999,24:181-185

[3] Li D, Roberts R: WD-repeat proteins: structure characteristics, biological function, and their involvement in human diseases[J]. Cell Mol Life Sci. 2001,58:2085-97

[4] Chernova OB, Hunyadi A, Malaj E et al: A novel member of the WD-repeat gene family, WDR11, maps to the 10q26 region and is disrupted by a chromosome translocation in human glioblastoma cells[J]. Oncogene 2001,20:5378-5392

[5] Honore B, Baandrup U, Nielsen S et al: Endonuclein is a cell cycle regulated WD-repeat protein that is up-regulated in adenocarcinoma of the pancreas[J]. Oncogene 2002,21,1123-1129

[6] van Nocker S, Ludwig P: The WD-repeat protein superfamily in Arabidopsis: conservation and divergence in structure and function[J]. BMC Genomics 2003,4:50

[7] Proikas-Cezanne T, Waddell S, Gaugel A, Frickey T, Lupas A, Nordheim A. WIPI-1alpha (WIPI49), a member of the novel 7-bladed WIPI protein family, is aberrantly expressed in human cancer and is linked to starvation-induced autophagy[J]. Oncogene 2004,58:9314-25

[8] Barth H, Meiling-Wesse K, Epple UD et al: Autophagy and the cytoplasm to vacuole targeting pathway both require Aut10p[J]. FEBS Lett 2001,508:23-28

[9] Georgakopoulos T, Koutroubas G, Vakonakis I, et al: Functional analysis of the Saccharomyces cerevisiae YFR021w/YGR223c/YPL100w ORF family suggests relations to mitochondrial/peroxisomal functions and amino acid signalling pathways[J]. Yeast 2001,18:1155-1171

[10] Guan J, Stromhaug PE, George MD, et al: Cvt18/Gsa12 Is Required for Cytoplasm-to-Vacuole Transport, Pexophagy, and Autophagy in Saccharomyces cerevisiae and Pichia pastoris[J]. Mol. Cell. Biol 2001,12:3821-3838

[11] Melendez A, Talloczy Z, Seaman M, et al: Autophagy Genes Are Essential for Dauer Development and Life-Span Extension in C. elegans[J]. Science 2003,301:1387-1391

[12] Gozuacik D and Kimchi A: Autophagy as a cell death and tumor suppressor mechanism[J]. Oncogene 2004,23:2891-2906

[13] Edinger ALand Thompson CB: Defective autophagy leads to cancer[J]. Cancer Cell 2003,4:422-424

[14] Kanehisa M, Bork P. Bioinformatics in the post-sequence era[J]. Nat Genet. 2003.33 Suppl:305-10

[15] Katoh M: Paradigm shift in gene finding method: from benchtop approach to desk-top approach[J]. Int J Mol Med 2002,10:677-682

[16] Masaru Katoh. Genome-Wide Search of Human Genes Implicated in WNT Signaling Pathway Using Bioinformatics[J]. Genome Informatics 2002.13:367-368

[17] Andrea Rossi1, Quinn Deveraux2, Boris Turk3, et al: Comprehensive search for cysteine cathepsins in the human genome[J]. Biol. Chem 2004,385:363-372

[18] Katoh M and Katoh M: Identification and characterization of mouse Erbb2 gene in silico[J]. Int J Oncol 2003,23:831-835

[19] Kim P, Kim N, Lee Y, et al: ECgene: genome annotation for alternative splicing[J]. Nucleic Acids Res 2005,33:D75-9

[20] Kent WJ: BLAT-the BLAST-like alignment tool[J]. Genome Res. 2002,12:656-64

[21] Rombel IT, Sykes KF, Rayner S, Johnston SA: ORF-FINDER: a vector for high-throughput gene identification[J]. Gene 2002,282:33-41

[22] Higgins, D., J. Thompson, T. Gibson, J. D. et al: CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice[J]. Nucleic Acids Res 1994,22:4673-4680

[23] Nicholas, K. B., H. B. Nicholas, Jr., and D. W. Deerfield II: GeneDoc: analysis and visualization of genetic variation[J]. EMBnew News 1997,4:14

[24] Nakai K, Horton P. PSORT: a program for detecting sorting signals in proteins and predicting their subcellular localization[J]. Trends Biochem Sci 1999,24:34-6

[25] McGuffin LJ, Bryson K, Jones DT: The PSIPRED protein structure prediction server[J]. Bioinformatics. 2000,16,404-405

[26] Kozak, M. An analysis of 5'-noncoding sequences from 699 vertebrate messenger RNAs[J]. Nucleic Acids Res. 1987,15:8125-48

[27] Lander ES, Linton LM, Birren B, et al: Initial sequencing and analysis of the human genome[J]. Nature 2001,409:860-921

[28] Ross MT, Grafham DV, Coffey AJ, et al: The DNA sequence of the human X chromosome[J]. Nature 2005,434:325-37

[29] Camargo AA, de Souza SJ, Brentani RR, et al: Human gene discovery through experimental definition of transcribed regions of the human genome[J]. Curr Opin Chem Biol 2002,6:13-6

[30] O'Brien KP, Tapia-Paez I, Stahle-Backdahl M, et al: Characterization of five novel human genes in the 11q13-q22 region[J]. Biochem Biophys Res Commun 2000,273:90-4

(上接第65页)

[25] Lejeune FJ, High dose recombinant tumor necrosis factor (rTNFα) administered by isolation perfusion for advanced tumors of the limbs: A model for biochemotherapy of cancer[J]. European J Cancer, 1995, 31A, 1009-1016

[26] Vaglini M, Belli F, Ammatuna M, et al. Treatment of primary or relapsing limb cancer by isolation perfusion with high-dose alpha-tumor necrosis factor, gamma-interferon, and malphalan[J]. Cancer, 1994, 73:483-492

[27] Fraker DL, Alexander HL, Andrich M, et al. Treatment of patients with melanoma of the extremity using hyperthermic isolated limb perfusion with melphalan, tumour necrosis factor, and interferon gamma: results of a tumor necrosis factor dose-escalation study[J]. J Clin Oncoligy. 1996,14:479-489

[28] Takahashi M, Fujimoto S, Kobayashi K, et al. Clinical outcome of intraoperative pelvic hyperthermo-chemotherapy for patients with Dukes' s C rectal cancer[J]. Int J Hyperthermia. 1994,10:749-754

[29] Sugiyama A, Katayama M, Matsuda T, et al. Hepatic arterial infusion chemotherapy combined with hyperthermia for metastatic liver tumors of colorectal cancer[J]. Seminars in Oncology. 1997,24:135-138

[30] storm FK, Baker H, Scanlon EF, et al. Magnetic-induction hyperthermia: Results of a 5-year multi-institutional national cooperative trial in advanced cancer patients[J]. Cancer, 1985,55:2677-2587

[31] Colombo R, Da Pozzo LF, Lev A, et al. A new approach using local combined microwave hyperthermia and chemotherapy in superficial transitional bladder carcinoma treatment[J]. J Uro, 1995,153:959-963

[32] Colombo R, Da Pozzo LF, Lev A, et al. Neoadjuvant combined microwave induced local hyperthermia and topical chemotherapy versus chemotherapy alone for superficial bladder cancer[J]. J Uro, 1996, 155:1227-1232

[33] Debes A, Willers R, Gobel U, et al. Role of heat treatment in childhood cancers: distinct resistance profiles of solid tumor cell lines towards combined thermochemotherapy[J]. Pediatr-Blood-Cancer, 2005,45(5):663-669

[34] Advani R, Saba HI, Tallman MS, et al. Treatment of refractory and relapsed acute myelogenous leukemia with combination chemotherapy plus the multidrug resistance modulator PSC 833[J]. Blood, 1999.93(3):787-795

[35] Stein U, Rau B, Wust P, et al. Hyperthermia for treatment of rectal cancer: Evaluation for induction of multidrug resistance gene(MDR1) expression[J]. Int J Cancer, 1999,80(1):5-12

[36] Kim SH, Yeo GS, Lim YS, et al. Suppression of multidrug resistance via inhibition of heat shock factor by querecetin in MDR cells[J]. Exp Mol Med, 1998,30(2):87-92

[37] 宋杨. 应用ZME-Ⅱ型大功率微波热靶点效应治疗肿瘤的临床疗效研究. 生物磁学,2005,5(4):16-18